A Study of motor control in the Cat Jaw muscles.

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A STUDY OF MOTOR CONTROL IN THE CAT JAW MUSCLES

by

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ABSTRACT

The behaviour of cat jaw-closing muscle spindles has been studied in anaesthetized animals during the application of passive stretch and in conscious animals during normal masticatory movements.

Two complimentary investigations were made. One concerned the measurement of some simple mechanical properties of these muscles and an examination of their histochemistry. In the second the functional components of the mesencephalic nucleus of the trigeminal nerve, which contains the first order cell bodies of the jaw muscle spindle afferents, were analyzed.

The jaw-closing muscles were found to be fast, having isometric twitch times of 11-13 msec. Tetanus:twitch ratios were high and the maximal rate of development of tetanic tension was reached at frequencies of stimulation of 300-500/sec. During repetitive stimulation, 75% of force was lost rapidly and thereafter a more gradual decline took place. Three histochemical fibre types were identified, corresponding essentially to A, B and C fibres of cat gastrocnemius, although differing in their relative numbers.

Two types of unit were found in the mesencephalic nucleus, namely jaw muscle spindle units and dental mechanoreceptor units. The latter were associated with different teeth, showed a range of adaptation and directional sensitivity to tooth pressure, and were concentrated in the caudal part of the nucleus.

Spindle units were distributed throughout the nucleus but were not segregated according to muscle of origin. Attempts to classify them into primary and secondary afferents were unsuccessful in the absence

of fusimotor excitation. However, following the administration of suxamethonium these units fell into two groups on the basis of dynamic sensitivity.

In conscious animals maximal frequencies occurred during lengthening, whilst shortening was accompanied by a partial or complete suppression of discharge. Units could be categorized as "high" or "low" frequency. The former were very dynamically sensitive, whereas the latter were more static in their responses.

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Some parts of the work reported in this thesis were carried out in collaboration with M.A. Bosley, R.W.H. Lee and A. Taylor. In all cases a substantial part of the experiments was the work of the author, who also performed most of the data processing.

Prof. A. Taylor.

(supervisor)

GENERAL INTRODUCTION

In spite of considerable advances in muscle spindle physiology (see Matthews, 1972) the fundamental questions concerning the use made by the central nervous system (CNS) of proprioceptive information remain largely unanswered. Consequently the role of spindles in motor regulation is speculative.

The participation of muscle receptors in the stretch reflex has long been recognised as a possible basis for the maintenance of muscle tone (Creed, Denny-Brown, Eccles, Liddell & Sherrington, 1932). Spindles have also been implicated in kinaesthesia (Sherrington, 1900) and in the learning of motor tasks (Buchwald, Stanish, Eldred, Gamble & Halas, 1963). However, the idea that has gained the greatest acceptance is that muscle spindles provide feedback in a servomechanism controlling contraction (Merton, 1951; 1953).

Evaluation of these theories ideally requires the recording of spindle activity, in conscious animals, during the performance of natural movements. The first experiments of this kind have recently been initiated in human subjects, in which percutaneous microelectrode recording has been made from hand muscle nerves during limited types of contraction (Hagbarth & Vallbo, 1969; Vallbo, 1971; 1973). The results so far obtained do not appear to be totally compatible with the proposed action of spindles in servo control.

Obviously there is considerable demand for similar sorts of observations in a variety of different situations in which a larger repertoire of movements can be studied.

Several years ago, with this aim in mind, Taylor & Davey (1968) saw the potential of the jaw muscle preparation. Anatomically the first order cell bodies of muscle spindle afferents from masseter, medial pterygoid and temporalis are located in the mesencephalic nucleus of the trigeminal nerve (MeNV; Corbin & Harrison, 1940; Jerge, 1963). In this situation these cells are accessible to microelectrode recording with a minimum of operative interference (Taylor & Davey, 1968; Cody, Lee & Taylor, 1972), and prolonged recording should be possible in the intact animal during a wide range of natural movements.

Unfortunately, little quantitative information is available of the properties of the jaw muscles in the cat. Furthermore, considerable controversy surrounds the subject of proprioceptive innervation of the cranial region (Hosokawa, 1961).

The initial stages of the present work were undertaken to provide background knowledge of these muscles and to resolve some of the discrepancies in the findings of previous authors.

Section 1 deals with the measurement of some simple mechanical properties of the jaw-closing muscles. An accompanying histochemical study was made.

Section 2 concerns the determination of the cell types present in the MeNV. It was thought especially important to find out whether, in addition to jaw muscle spindle afferents (Corbin & Harrison, 1940), tendon organ afferents (Smith, 1969) or extraocular muscle stretch receptor afferents (Fillenz, 1955) were represented. Obviously the presence of the latter two cell types could lead to

confusion during recording in the conscious animal, when opportunities for appropriate testing are limited.

Section 3 describes attempts functionally to subdivide spindle units into groups corresponding to primary and secondary afferents.

Finally in Section 4 experiments in conscious animals are considered in which spindle activity was recorded during eating, drinking and related movements.

SECTION 1

MECHANICAL AND HISTOCHEMICAL PROPERTIES OF THE CAT JAW MUSCLES

1.1 INTRODUCTION

Recent knowledge of single motor unit properties points to certain general principles in the functional organization of mammalian skeletal muscle.

Henneman (1968) incorporated evidence of variations in the size, excitability, speed and fatiguability of individual motor units of cat hindlimb muscles into an overall plan of motor unit coordination in muscle contraction. According to this scheme the motor units of a given muscle are recruited in a set order, under all circumstances, governed by the excitability of their motorneurones. Since small motor units are most excitable these are the first to be activated and are thus subject to greatest usage. These units, in addition, are slowly contracting and resistant to fatigue. Such properties suit them well to the modest sustained contractions demanded in postural regulation and also to fine precisely controlled movements. Conversely the least excitable motorneurones are associated with large, rapidly contracting motor units which, broadly speaking, are more susceptible to fatigue. These units would be better suited to large, phasic contractions.

Alongside the development of this functional picture considerable evidence has been accumulated to suggest that the contractile properties of motor units are related to the histochemical characteristics of their constituent fibres. In particular, the application of enzymological methods has demonstrated differences between muscle fibres and the possibility that fibres may be divided into a number of categories.

Indirect evidence from whole muscle and cross-innervation studies indicates that the speed of contraction of individual fibres may be related to their myosin adenosine triphosphatase (MATPase) activity, whilst mitochondrial oxidative enzymes may be important in determining fatiguability.

Recently direct evidence has been supplied by Burke and coworkers (1971; 1973a; 1973b) from experiments on single motor units in which both mechanical and histochemical properties were studied. In these experiments the majority of motor units could be allocated to one or other of three distinct groups, corresponding to the three basic fibre types.

The jaw-closing muscles, masseter, pterygoid and temporalis, are known to be very fast, approaching the extraocular muscles in their speed of contraction (Taylor & Davey, 1968). These muscles therefore make an interesting comparison with the slower limb muscles. The only previous report of jaw muscle histochemistry was in the rat (Hiiemae & Houston, 1971). However species differences are known to exist between fibre types in the cat and rat (see Yellin & Guth, 1970). In addition this study was based exclusively on Sudan black staining, which did not permit reliable classification of fibres and no accompanying physiological recording was made.

In the present work the possibility that some of the above generalisations, relating histochemistry to function, also extend to the jaw muscles, has been tested. A number of simple contraction characteristics, e.g. speed of contraction, $^{tet}/_{tw}$ ratio and fatiguability were measured under isometric conditions. Also muscle samples were stained to demonstrate MATPase and succinic dehydrogenase (SDH) activity in addition to glycogen and lipid content.

1.2 HISTORICAL REVIEW

FIBRE TYPES, MOTOR UNITS AND THE CORRELATION OF HISTOCHEMISTRY AND FUNCTION IN SKELETAL MUSCLES

As long ago as 1678 Stefano Lorrenzini commented upon striking differences in the colour of various rabbit limb muscles. Kuhne (1865) showed these colour differences to be a characteristic of the fibres themselves, rather than of their blood content as had previously been supposed.

Ranvier (1873, 1874), in the first of a classical series of experiments on rabbit red and pale muscles, demonstrated the former to be more slowly contracting and to have a lower tetanic fusion stimulation frequency. Soon afterwards, he found red muscles to be more resistant to fatigue than pale ones (Ranvier, 1880). Furthermore Ranvier drew attention to an association between functional differences and histological structure, the red muscles having greater amounts of granular sarcoplasm and more marked longitudinal striations.

These general principles were confirmed by a number of subsequent workers (e.g. Kronecker & Sterling, 1878; Hay, 1901; Fischer, 1908; Lee, Guenther & Meleney, 1916; Denny-Brown, 1929).

However evidence from other studies indicated that this scheme, equating redness with slowness of contraction, was an oversimplification and that the gross colour of a muscle was less important in determining its contraction properties than had been initially believed.

Knoll (1891) found that some red muscles contracted more quickly than pale ones and Paukul (1904), studying a range of rabbit muscles, concluded that whilst all slow muscles were red (e.g. soleus), not all red muscles were slow (e.g. masseter).

These results fitted well with those of Meyer (1875) who had earlier found that the histological features of the rabbit masseter resembled those commonly present in pale muscles. Further histological studies revealed that the majority of mammalian muscles were not homogeneous, but consisted of a mixture of different types of muscle fibres. Most authors recognised two basic categories of fibres. "Red" fibres being dark and containing many mitochondria whilst "pale" fibres were clear and had few mitochondria (Grutzner, 1884; Schaffer, 1893; Bullard, 1919; Denny-Brown, 1929).

This led Grutzner (1884) to postulate that the muscles of higher mammals were composed of an intimate combination of rapidly and slowly contracting fibres. In retrospect it is interesting to note that this hypothesis was refuted by Denny-Brown (1929).

As a result of subsequent work in a variety of muscles it is now generally accepted that muscle fibres may be broadly divided into three categories according to their histochemistry (see Close, 1972). In spite of this the subject of histochemical classification of muscle fibres remains extremely complex and often controversial. A brief outline of the principal classifications employed at the present time is given in Appendix A.

Since Ranvier's experiments a good deal of effort has been devoted to the recording of contraction properties of a variety of whole muscles. This has emphasized individual differences in such parameters as twitch speed, tetanic fusion frequency, $^{Tet}/_{Tw}$ ratio, rate of rise of tetanic tension and fatiguability, even within particular muscle groups, e.g. cat triceps surae (see Cooper & Eccles, 1930;; Buller & Lewis, 1965). The general principles expounded by Ranvier concerning speed, tetanic

fusion frequency and fatiguability have been broadly supported and extended. It is now recognized that fast muscles generally have higher $^{Tet}/_{Tw}$ ratios, e.g. eye muscles (Bach-y-Rita & Ito, 1966), and that increasing stimulation frequency above that required for tetanic fusion results in a more rapid development of tension (Buller & Lewis, 1965).

However, attention has been progressively drawn to the study of single motor units. Eccles & Sherrington (1930) calculated the extent of innervation of a single motor axon by comparing, in de-afferented hindlimb preparations, the numbers of efferent nerve fibres and muscle fibres although at the time they did not appreciate the significance of γ fibres. Later Sherrington wrote "a muscle with its motor nerves may be thought of as an additive assemblage of motor units". Over the last ten years, conscious of the presence of three principal histochemical fibre types, many workers have concentrated on characterizing three corresponding groups of motor units.

Predictably, in muscles composed entirely, or almost entirely, of one histochemical fibre type single populations of motor units, according to speed, tetanic tension and fatiguability, have been found, e.g. cat soleus (McPhedran, Weurker & Henneman, 1965). Thus the so-called "intermediate"* fibres (Close, 1972) of cat soleus correspond to slow twitch, unfatiguing motor units as would be anticipated from measurements on the whole muscle. This encourages the assumption that in the absence of direct single motor unit records, the properties of the whole muscle

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* Although often referred to as "intermediate" the fibres of cat soleus show certain histochemical differences from the "intermediate" fibres of mixed muscles, e.g. gastrocnemius (see Appendix A).

represent a reasonable approximation to those of individual units and their corresponding histochemical fibres. It seems likely therefore that the "red" fibres of the rapid rabbit thyroarytenoid (Hall-Craggs, 1968) form fast twitch motor units whilst the "intermediate" fibres of the guinea-pig soleus (Barnard, Edgerton, Furukawa & Peter, 1971) constitute slow-twitch units.

However, in mixed muscles the evidence from earlier single motor studies was far less clear cut. In no instance did the measurement of twitch speed or tetanic tension reveal more than two groups of motor units despite the presence of three types of muscle fibre, e.g. cat respiratory muscles (Andersen & Sears, 1964), cat gastrocnemius (Wenker, McPhedran & Henneman, 1965), cat flexor hallucis longus (FHL, Olson & Swett, 1966). This prompted the suggestion that in mixed muscle both "red" and "white" fibres must form fast motor units and indeed Olson & Swett (1966), in FHL, found that the fast units showed variation in isometric tetanic tension and fatiguability. These workers proposed that the larger fast units which were susceptible to fatigue corresponded to "white" fibres whereas those resistant to fatigue corresponded to "red" fibres. Further indirect supporting evidence was provided by Close's (1967) findings in the fast rat extensor digitorum longus (EDL) that motor units showed a narrow range of twitch speeds although the muscle comprises "white" and "red" fibres in almost equal proportions (Edgerton & Simpson, 1969).

The introduction by Edström and Kugelberg (1968(a), (b)) of a technique for the identification of motor units, in histochemical preparations, by the in vivo depletion of their glycogen content by prolonged electrical stimulation, has formed the basis of recent direct observations. Their preliminary experiments in the rat tibialis anterior confirmed

the long suspected concept that motor units are composed of one histochemical fibre type and that constituent fibres are scattered diffusely throughout the muscle.

Taking advantage of this technique Burke, Levine, Zajac, Tsairis & Engel (1971), Burke, Tsairis, Levine, Zajac & Engel (1973) and Burke, Levine, Tsairis & Zajac (1973) directly correlated physiological and histochemical characteristics in motor units of the cat triceps surae. They identified three types of motor units using a combination of physiological parameters, e.g. twitch speed, tetanic tension, "sag" in unfused tetani and sensitivity to fatigue. Subsequent histochemical mapping showed the histochemical profiles of these three groups to correspond to the three fibre types. Motor units designated FF (fast contracting, fast fatiguing) had high MATPase and low mitochondrial oxidative enzyme activity; FR (fast contracting, fatigue resistant) units stained strongly for MATPase and moderately for oxidative enzymes; S (Slowly contracting, fatigue resistant) units were poor in MATPase and rich in oxidative enzymes. Thus FF, FR and S motor units corresponded to "white" (type A*), "red" (type C*) and "Intermediate" (type B*) fibres respectively.

Recently Kugelberg (1973) has completed a comparable study in rat hindlimb muscles. Although similar rules relating speed and fatigability of units to their enzymology apply, interpretation is complicated by additional subdivision of fibre types.

The combination of physiological and histochemical evidence suggests that the speed of contraction of a motor unit is related to the MATPase activity of its constituent muscle fibres whilst mitochondrial content is important in determining fatiguability.

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* Yellin & Guth (1970) classification.

This hypothesis has received considerable support from a variety of other sources.

Cross-innervation studies in several species have shown that both the speed of contraction and number of fibres with high MATPase activity of a slow muscle, e.g. soleus, increases following implantation of a fast muscle nerve, e.g. tibial (see Yellin, 1967). Comparable work in developing mammalian muscle, in which speed may increase with maturation, has been complicated by the differing pH stability of neonate and adult MATPases, so that histochemical techniques, at pH 9.4, cannot readily be applied (Guth & Samaha, 1972).

Also parallel biochemical estimates from whole muscles provide valuable additional data. Buller, Mommaerts & Seraydarian (1969) found that the specific activity of MATPase of fast cat muscle (FDL) decreased after innervation by slow soleus nerve to almost the same level as in normal soleus. Guth & Samaha (1972), in developing rabbit muscles, demonstrated that increased MATPase activity accompanied speeding of the muscles.

1.3 METHODS

1.3.1 Mechanical Recording

Six adult cats, of either sex, weighing 1.5 - 3.0 kg, were used. Animals were anaesthetized with pentobarbitone sodium (60 mg/kg I.P.) and maintained at a deep level by additional intravenous supplements.

Since the jaw muscles are mechanically complex, with fibres varying in orientation and lacking simple tendinous origins or insertions, it was impractical to record from whole muscles. Instead isometric contractions were recorded from strips of masseter and temporalis. The muscles were separated from the bone at their upper end, i.e. origin, and divided by a series of incisions running parallel to the orientation of the fibres. These cuts were made at intervals of approximately 3 mm, being careful to leave the blood supply as intact as possible. Small loops of non-compliant thread or wire were sewn into the free ends of such strips and connected to a strain gauge (Statham ± 24 oz) mounted in line with their direction of contraction.

Occasionally small flakes of bone, with the muscle still attached, were removed and connexion made to these. This reduced the tendency for the thread to tear out or become detached during strong contractions. A skin pool filled with liquid paraffin surrounded the muscles and was maintained at 37-38°C. The output of the strain gauge was displayed on an oscilloscope (Tektronix Inc., type 565) for photography.

1.3.2 Electrical Stimulation

Muscles were stimulated by means of indwelling enamelled silver wires with their final 2 mm bared. These wires were connected to an isolated

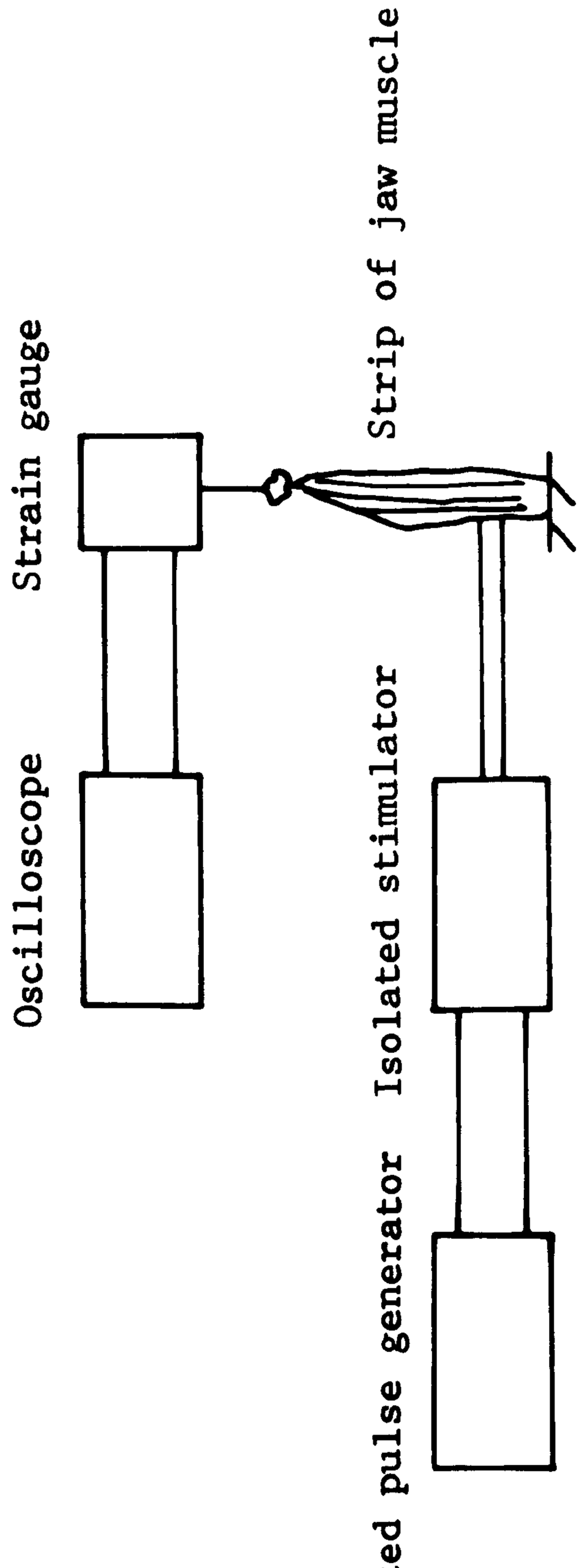

Fig. 1.1 GENERAL EXPERIMENTAL SET-UP.

stimulator (Devices Ltd., Mk IV) which allowed the amplitude and duration of stimuli to be adjusted. The isolated stimulator was triggered by gated pulses applied at periodic intervals by a second pulse generator (Devices Ltd., Digitimer).

The optimal length for the development of tension by individual strips for twitch contractions using supramaximal stimulation was initially determined and kept constant throughout.

For each muscle strip five to ten single muscle twitches and a series of tetanic contractions of 500 msec duration at increasing frequencies of 20-200/sec (pulse width 0.2 msec) were routinely recorded. Also, in a number of strips, the rate of rise of tetanic tension was measured at frequencies up to 600/sec.

The fatiguability of jaw muscles was assessed by the application of trains of stimuli (pulse width 0.2 msec) at 60-90/sec, lasting 330 msec, every 1 min. Stimulation frequency for a given jaw muscle strip was chosen to correspond to that used in recent single motor unit studies in the cat hindlimb muscles (Burke, Levine, Tsairis & Zajac, 1973).

$$\text{Frequency of stimulation (ips)} = \frac{Tp(\text{gastrocnemius})}{Tp(\text{jaw muscle strip})} \times 40$$

where Tp is the time to peak isometric twitch tension in msec and 40 i.p.s. was the frequency of stimulation used by Burke and coworkers in the cat gastrocnemius.

1.3.3 Photography

Force records were photographed from the oscilloscope with a continuous recording camera (Nihon Kohden Kogyo Co. Ltd., type PC-2H) using 35 mm film (Ilford Ltd., 5B52) or recording paper (Kodak Ltd., RP30).

1.3.4 Histochemistry

Jaw muscle specimens were obtained from eight adult cats, of either sex, weighing 1.5-2.0 kg. Under pentobarbitone sodium anaesthesia small pieces of masseter, pterygoid and temporalis, as well as gastrocnemius and occasionally soleus, were removed from several sites in each muscle. Each sample was blotted and immediately frozen by immersion in liquid nitrogen-cooled isopentane at about -160°C. Subsequently 10μ transverse sections were cut on a cryostat (Pearse cold microtome, Slee)at -15°C to -20°C.

All sections were stained for MATPase (Padykula & Herman, 1955), SDH (Stein & Padykula, 1962), glycogen (P.A.S. reaction, McManus, 1946) and lipid (Sudan black). Details of these methods are given in Appendix B.

Specimens were examined microscopically. The intensity of histochemical reactions was graded by eye and in the cases of SDH and Sudan black staining the cytological distribution of granules noted.

Fibre cross-sectional areas in sections treated to demonstrate MATPase and SDH activity, were estimated from diameters measured using an eye piece micrometer. For this purpose the mean of the largest diameter and that at right-angles to it was taken.

It often proved possible to identify the same groups of fibres in each of a stained series of sections of a given sample. This permitted a direct comparison of the reactions of individual fibres to the range of histochemical techniques employed.

1.4 RESULTS

1.4.1 Mechanical Properties

Fig. 1.2 shows isometric twitches of the jaw-closing muscles, in response to single supramaximal electrical stimuli, to be fast. The mean times to peak twitch tension (Tp)* for strips of masseter and temporalis muscles were 13.1 (SD 2.27, n = 18) msec and 11.4 (SD 2.11, n = 21) msec respectively. The corresponding times to half relaxation ($T_{\frac{1}{2}R}$)* were 12.8 (SD 2.49) msec and 9.81 (SD 1.84) msec.

Stimulation of these muscles at increasing frequencies produced complete tetanic fusion at about 100/sec (Fig. 1.3). Subsequently there was negligible additional development of tetanic tension.

However at higher frequencies the rate of rise of tetanic tension ($\frac{dP_{tet}}{dt}$) continued to increase as may be seen in Fig. 1.4. Over the range 100 to 200/sec $\frac{dP_{tet}}{dt}$ rose linearly with log frequency (Fig. 1.5). Little further increase in $\frac{dP_{tet}}{dt}$ occurred between 300 and 500/sec and the maximal value of 4-5% maximum twitch tension (P_o)/msec, for both masseter and temporalis, was generally obtained at about 400/sec. In a few strips higher frequencies of stimulation were tried and at rates in excess of 600/sec there tended to be a reduction in $\frac{dP_{tet}}{dt}$.

The tetanus:twitch (Tet:Tw) ratios were high for these muscles being 7.79 (SD 2.40, n = 18) for masseter and 9.60 (SD 4.06, n = 20) for temporalis, as is illustrated in Fig. 1.6.

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* Tp is defined as the time from initial rise to the maximum twitch tension (P_o) and $T_{\frac{1}{2}R}$ as the time taken for P_o to fall to half its value.

Fig. 1.2

The time course of isometric twitches of strips of (a) masseter and (b) temporalis. Values of T_p were 10.0 and 11.5 msec. respectively.

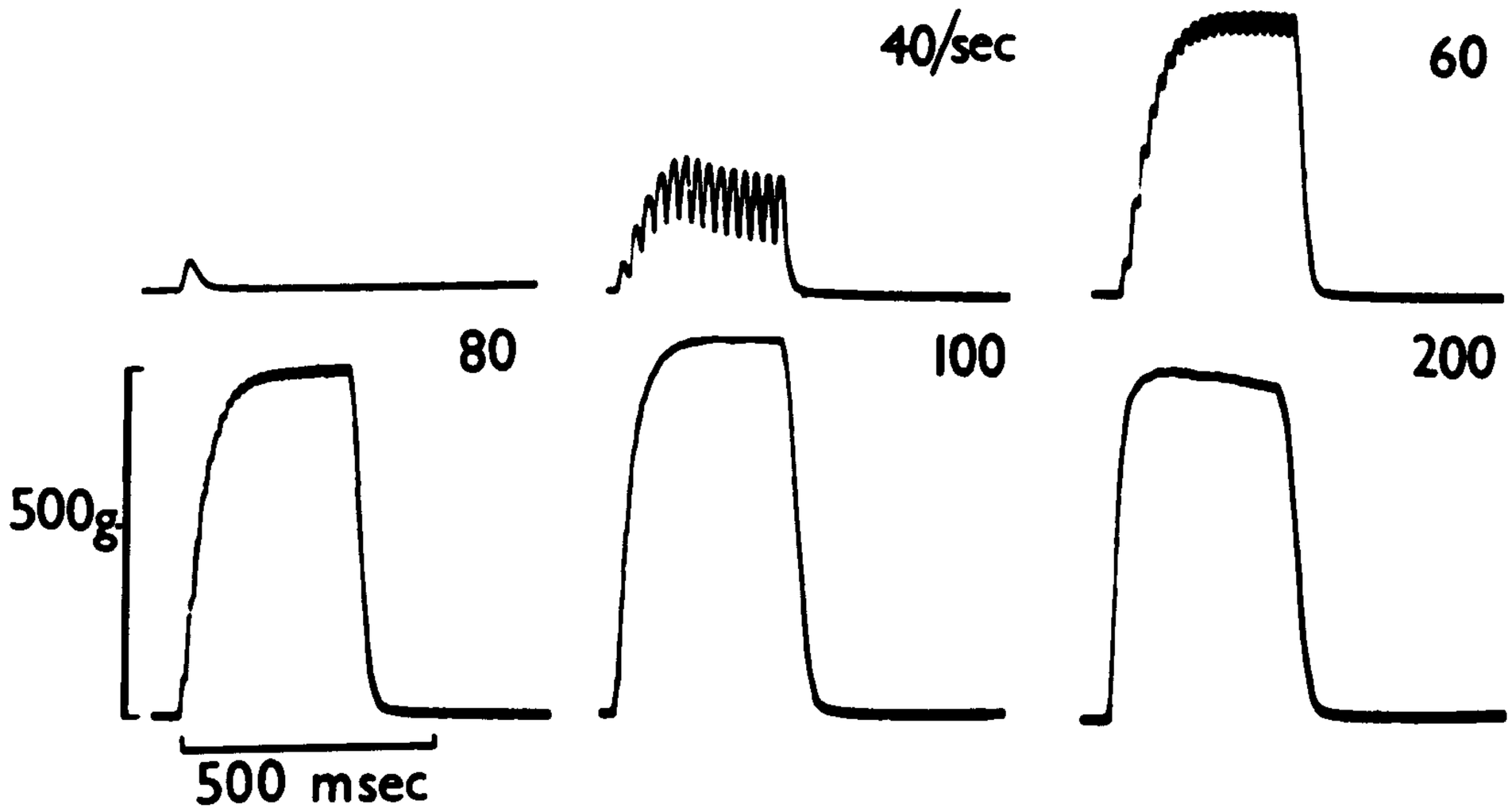

Fig. 1.3

Isometric tension produced by a strip of temporalis muscle during stimulation at increasing frequencies. Tetanic fusion was obtained at 100/sec.

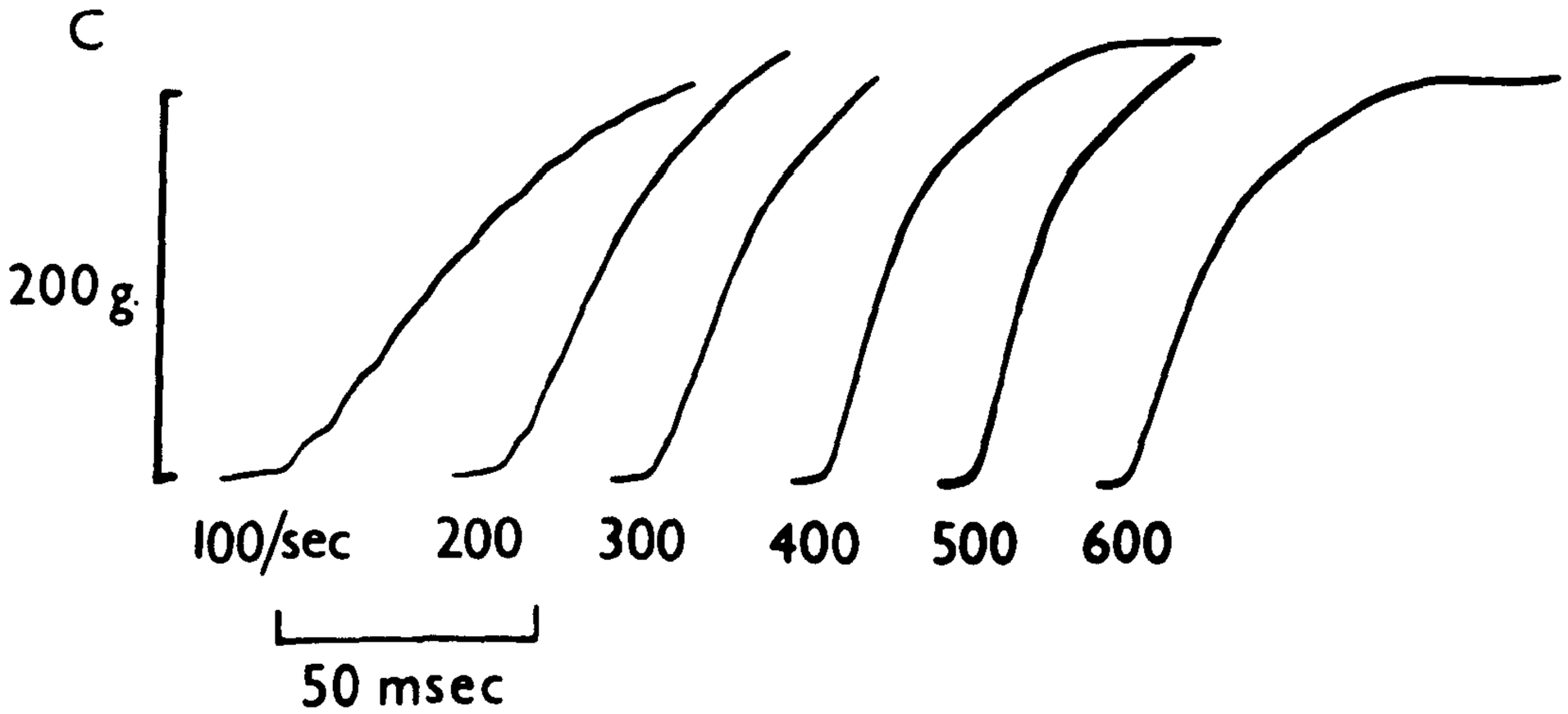

Fig. 1.4

The effect of increasing frequencies of stimulation on the rate of rise of tetanic tension of a strip of temporalis. A maximal dP_{tet}/dt of 4.6 $^{o}/o$ P_o/msec was obtained at 500/sec.

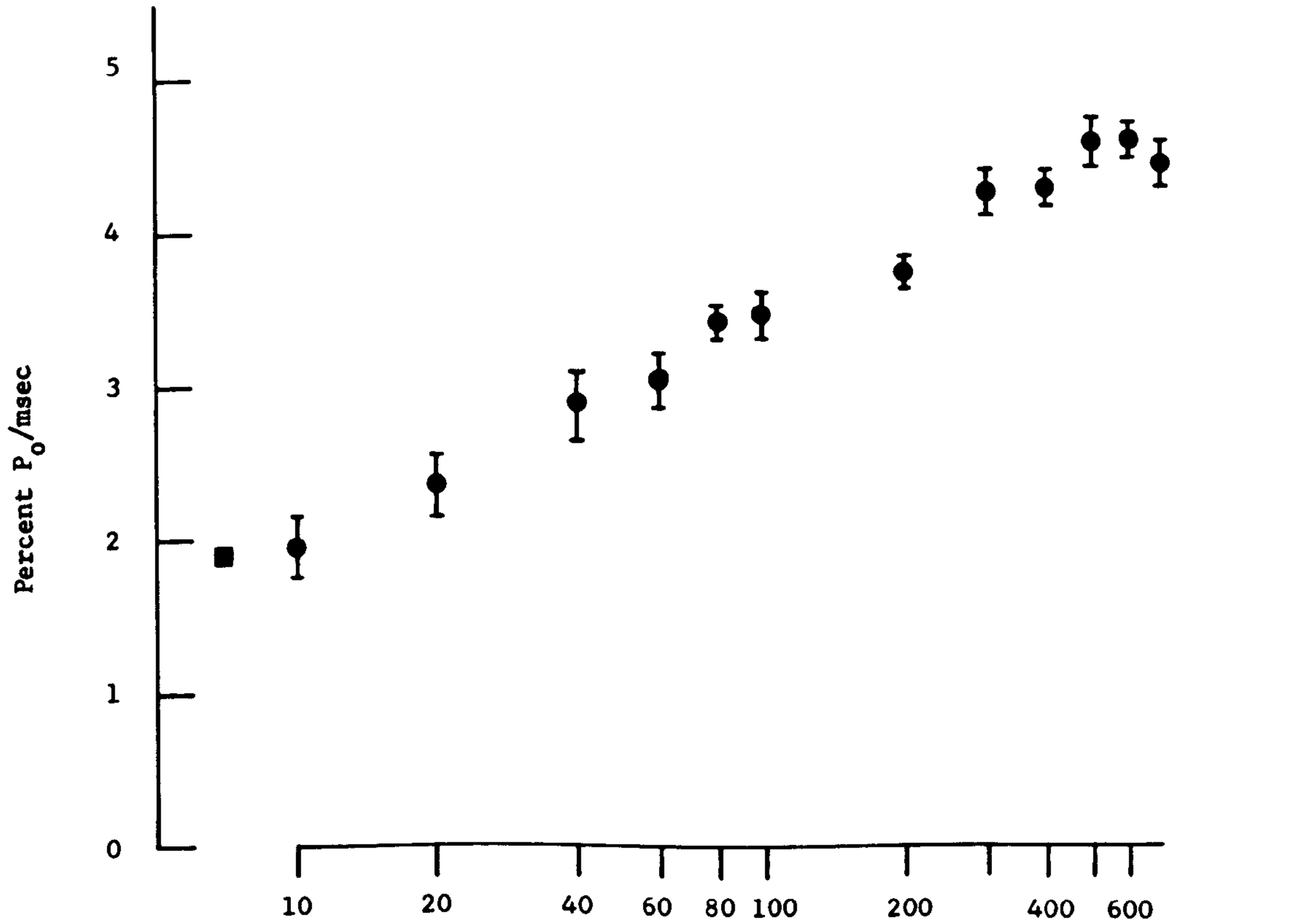

Fig. 1.5

The relationship between rate of rise of tetanic tension and frequency of stimulation for strips of jaw muscle (masseter and temporalis combined). Closed square represents the corresponding value for twitches. Bars are the standard errors of the mean.

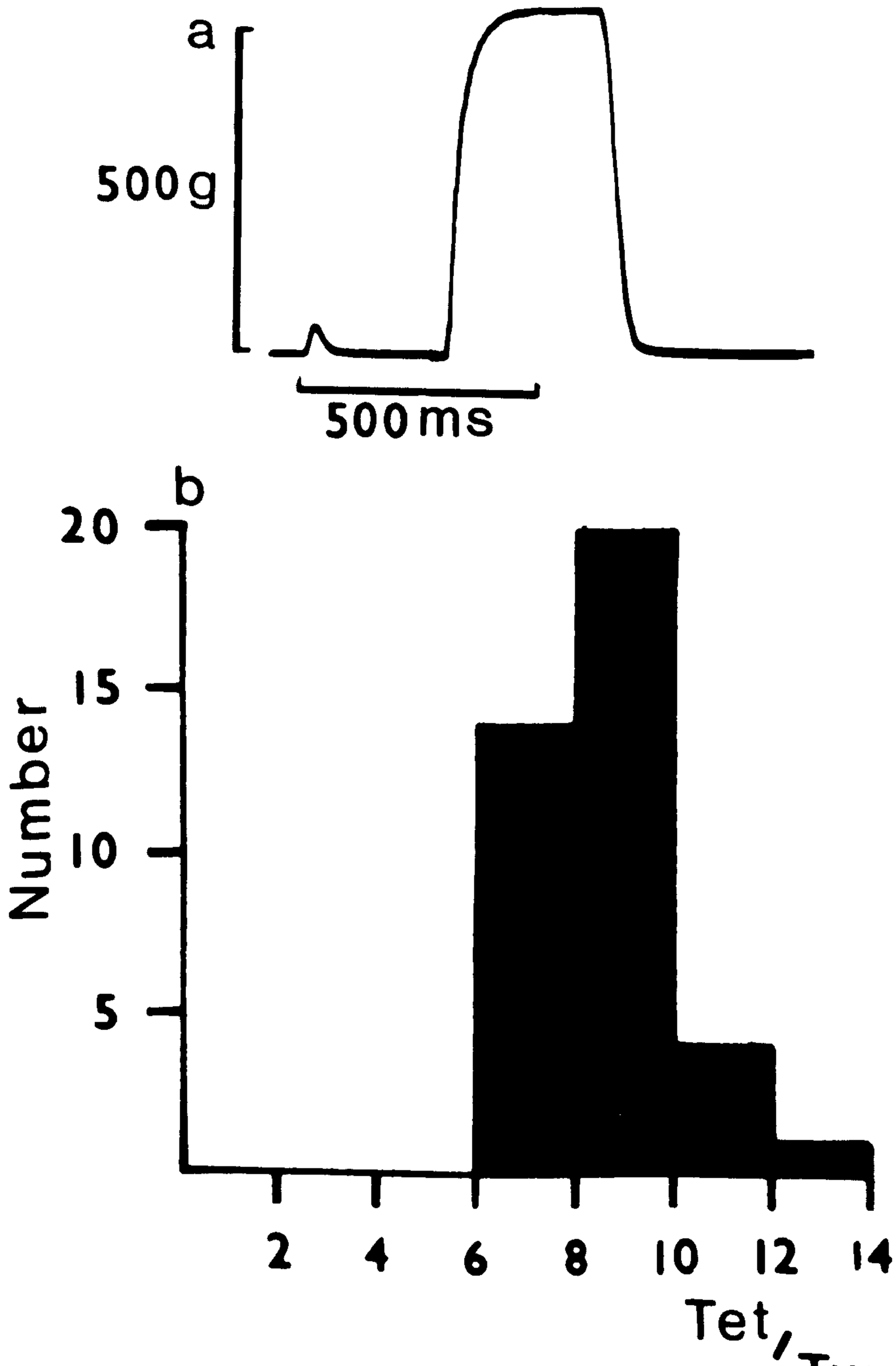

Fig.1.6 a Temporalis strip
b Pooled

No statistically significant differences were found for either contraction times or tet:tw ratios between these two jaw muscles, as judged by the t-test.

Fig. 1.7 shows the results of a study on the fatigue susceptibility of a representative jaw muscle strip during the application of repetitive trains of stimuli. A rapid fall in tetanic tension to about one-quarter of its initial value was consistently observed during the first minute of stimulation. Force continued to fall, more gradually, over the next 2-3 min, and by the end of five minutes up to nine-tenths of the original tetanic tension had been lost. The effects of more prolonged periods of stimulation were not tested. This pattern of fatigue was obtained in both masseter (6) and temporalis (5) strips. The early phase of rapid fatigue was particularly repeatable but more variation was found during the later stages of stimulation (Table 1.1). In some cases force had declined to almost zero by three minutes, possibly denoting an inadequate blood supply.

In none of the jaw muscles was there evidence of marked post-tetanic potentiation. Twitch tension measured 10 sec after tetani of 500 msec duration although normally greater than the pretetanic value (Fig. 1.8) was never more than 130%.

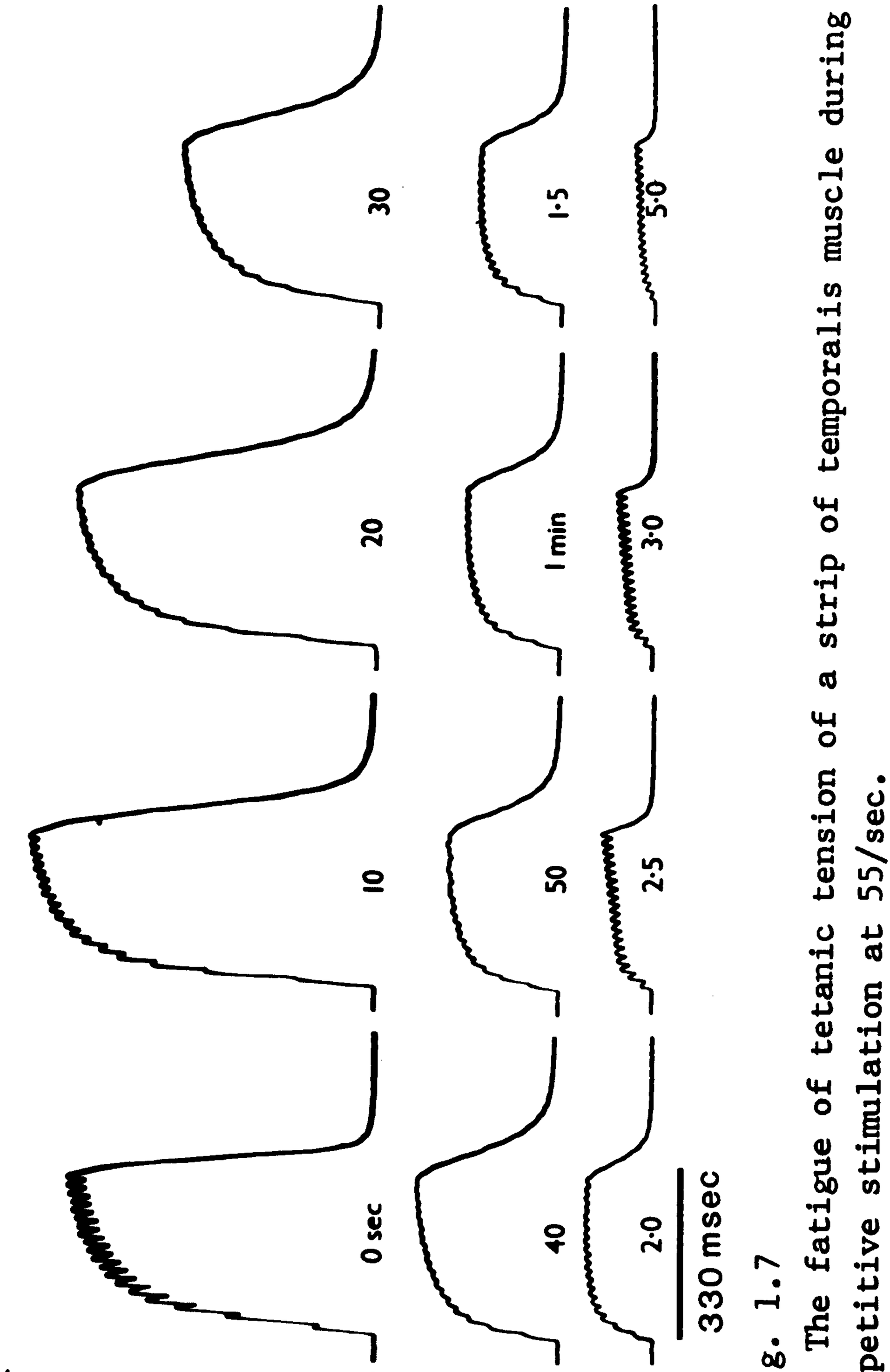

Fig. 1.7
The fatigue of tetanic tension of repetitive stimulation at 55/sec.

TABLE 1.1 FATIGUE OF TETANIC FORCE IN CAT JAW MUSCLE STRIPS (POOLED)

TIME (SEC.)	0	10	20	30	40	50	60	90	120	150	180	300
FORCE (normalized to value at time = 0 sec, mean and SD)	1 -	1.2 (±0.32)	0.98 (±0.21)	0.65 (±0.20)	0.52 (±0.16)	0.38 (±0.09)	0.31 (±0.04)	0.27 (±0.04)	0.24 (±0.06)	0.18 (±0.07)	0.13 (±0.07)	0.11 (±0.05)

N = 24

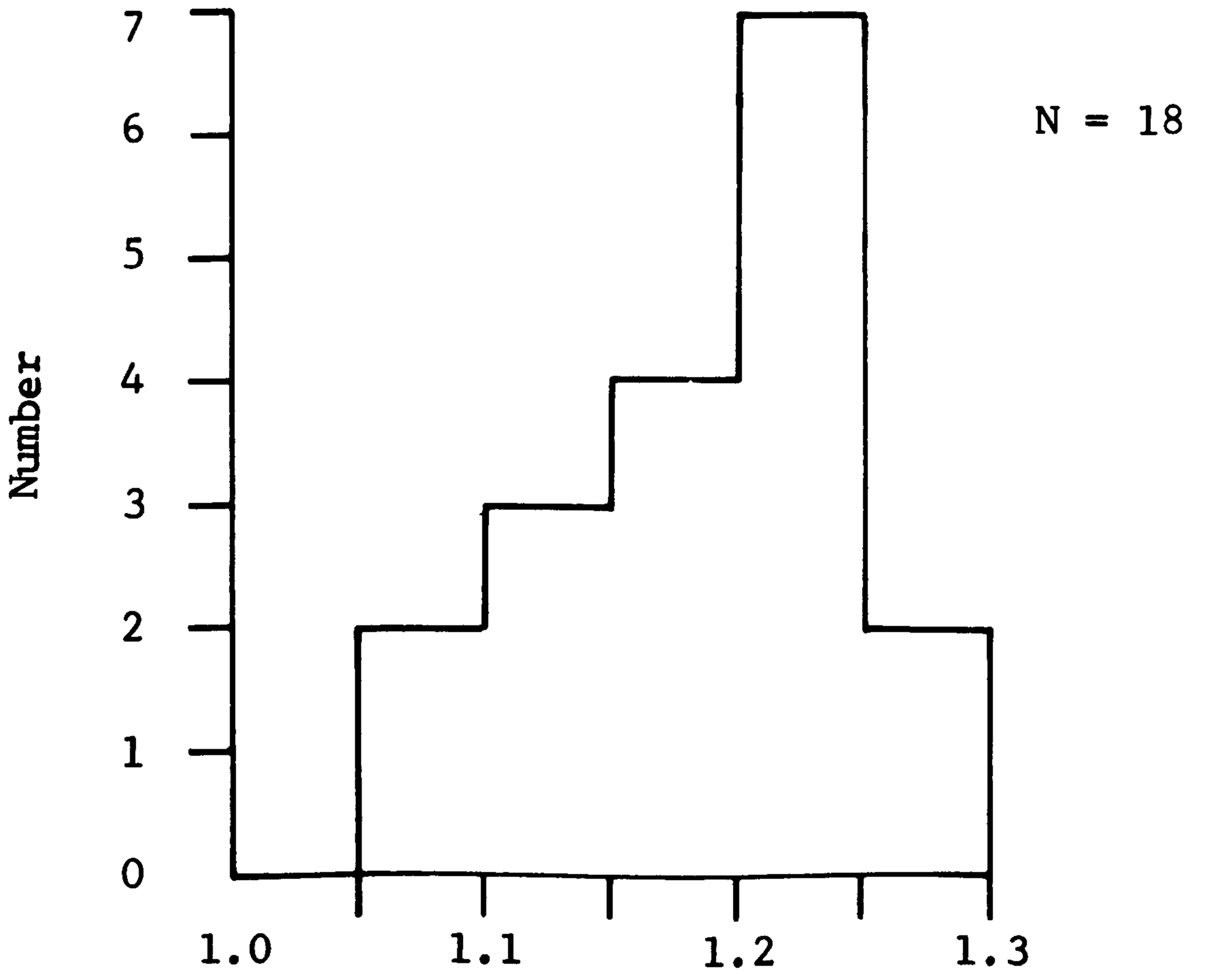

Fig. 1.8

Distribution of values of PTP in jaw muscle strips. Values are normalized to the pre-tetanic isometric twitch force.

1.4.2 Histochemical Properties

Three principal fibre types were identified in each of the cat jaw-closing muscles, according to a combination of their MATPase, SDH and PAS staining. These types essentially resembled the A, B and C fibres of cat mixed hindlimb muscles (e.g. gastrocnemius). However the jaw muscle fibre types showed some differences in their histochemistry from the corresponding types in limb muscles, particularly regarding MATPase staining and in their relative numbers.

Individual fibres were identified in adjacent serial transverse sections, stained in parallel for MATPase, SDH and glycogen. Photomicrographs of such sections (Figs. 1.9, 1.10 and 1.11) illustrate the histochemical profiles of fibres, respectively, from the masseter, pterygoid and temporalis muscles. Similar sections of gastrocnemius are included for comparison (Fig. 1.12). The classification of fibres, based primarily on SDH staining, is indicated.

In each of the jaw muscles the predominant type of fibre was large. MATPase activity was relatively strong, as judged by the density of staining, and even throughout the fibres. The SDH reaction was weak, diformazan particles being small and tending to be concentrated peripherally. This indicated a paucity of mitochondria, since SDH is almost exclusively confined to these organelles. The intensity of PAS staining pointed to the presence of a high glycogen content. This fibre type resembled the A fibres of gastrocnemius.

A second fibre type was of intermediate size. These fibres had MATPase activity comparable to the A fibres and were rich in glycogen. However SDH staining was strong. Coarse diformazan particles were located mainly at the periphery of the fibres where they formed a

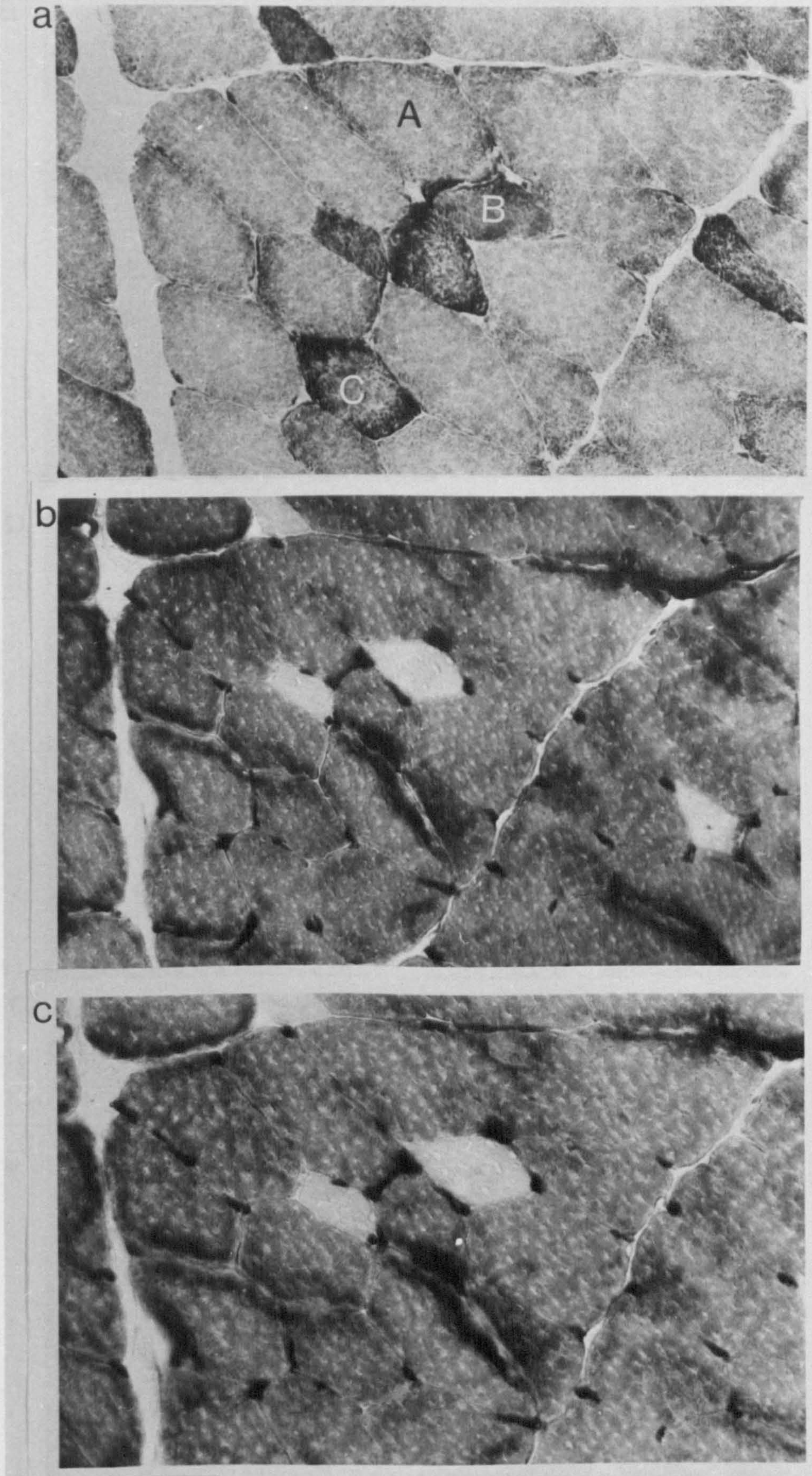

Fig. 1.9

The histochemical profiles of individual fibres identifiable in serial sections of masseter muscle stained for (a) SDH, (b) MATP'ase and (c) PAS. Calibration bar is 100μm.

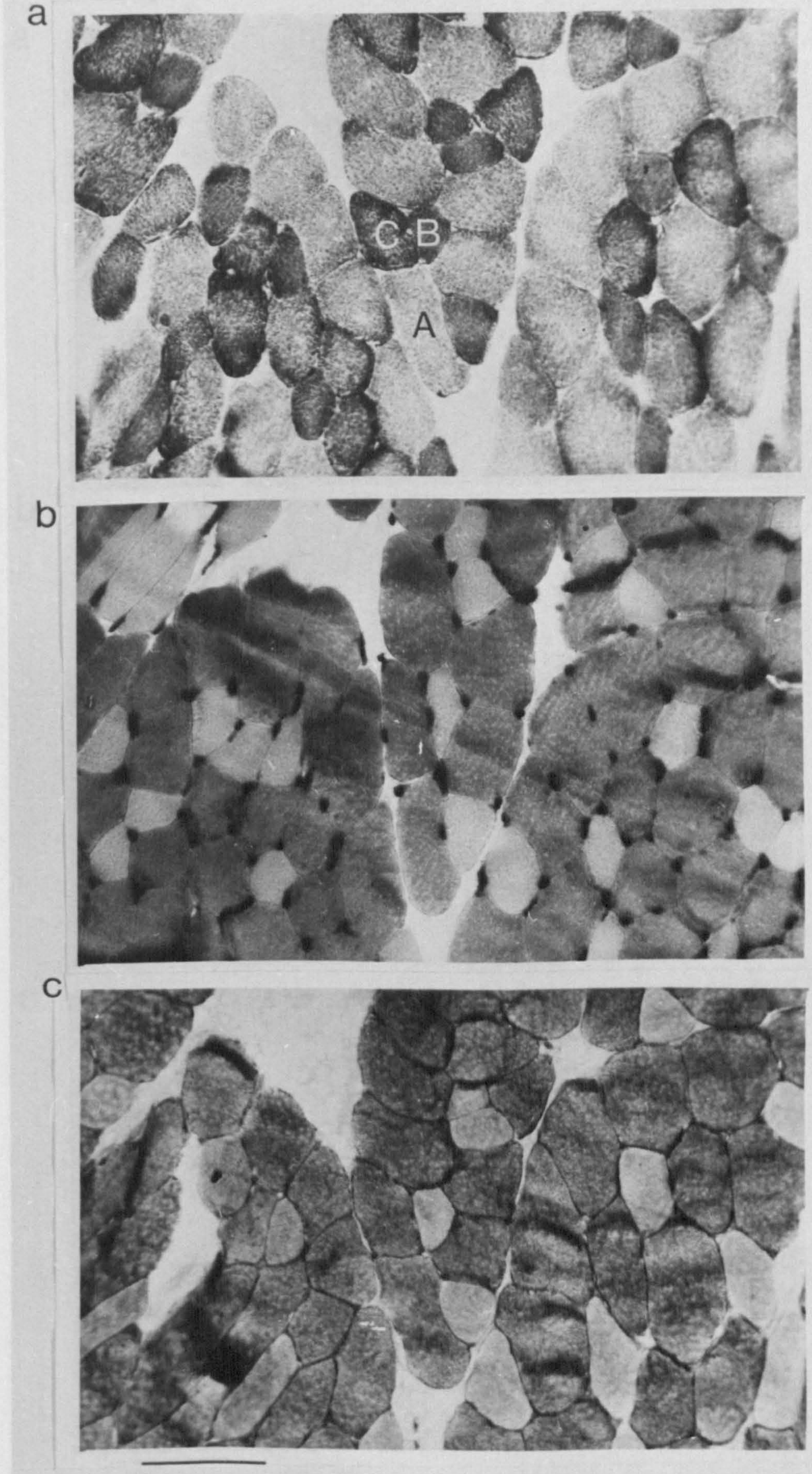

Fig. 1.10

The histochemical profiles of individual fibres identifiable in serial sections of pterygoid muscle stained for (a) SDH, (b) MATP'ase and (c) PAS. Calibration bar is 100μm.

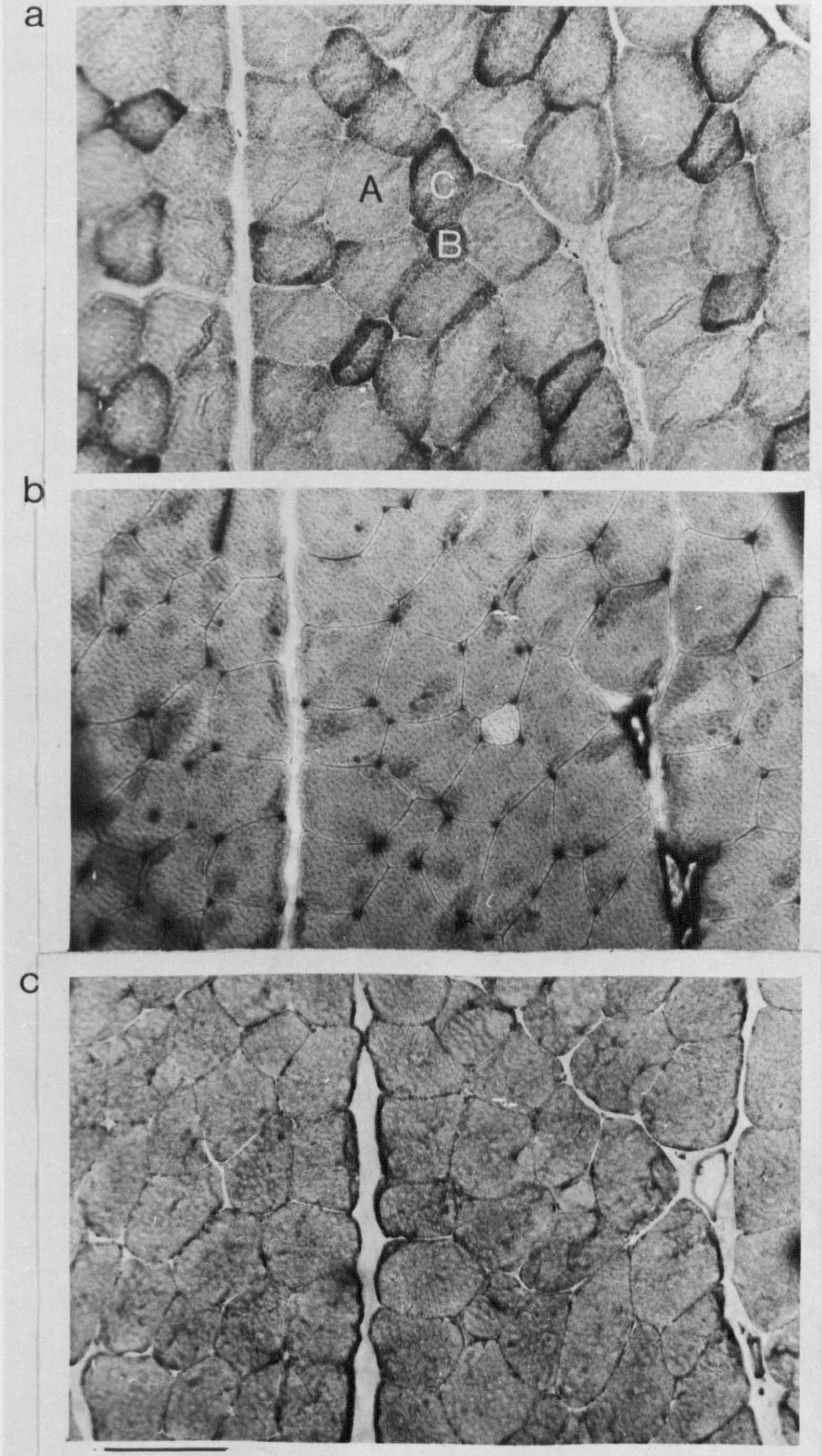

Fig. 1.11

The histochemical profiles of individual fibres identifiable in serial sections of temporalis muscle stained for (a) SDH, (b) MATP'ase and (c) PAS. Calibration bar is 100μm.

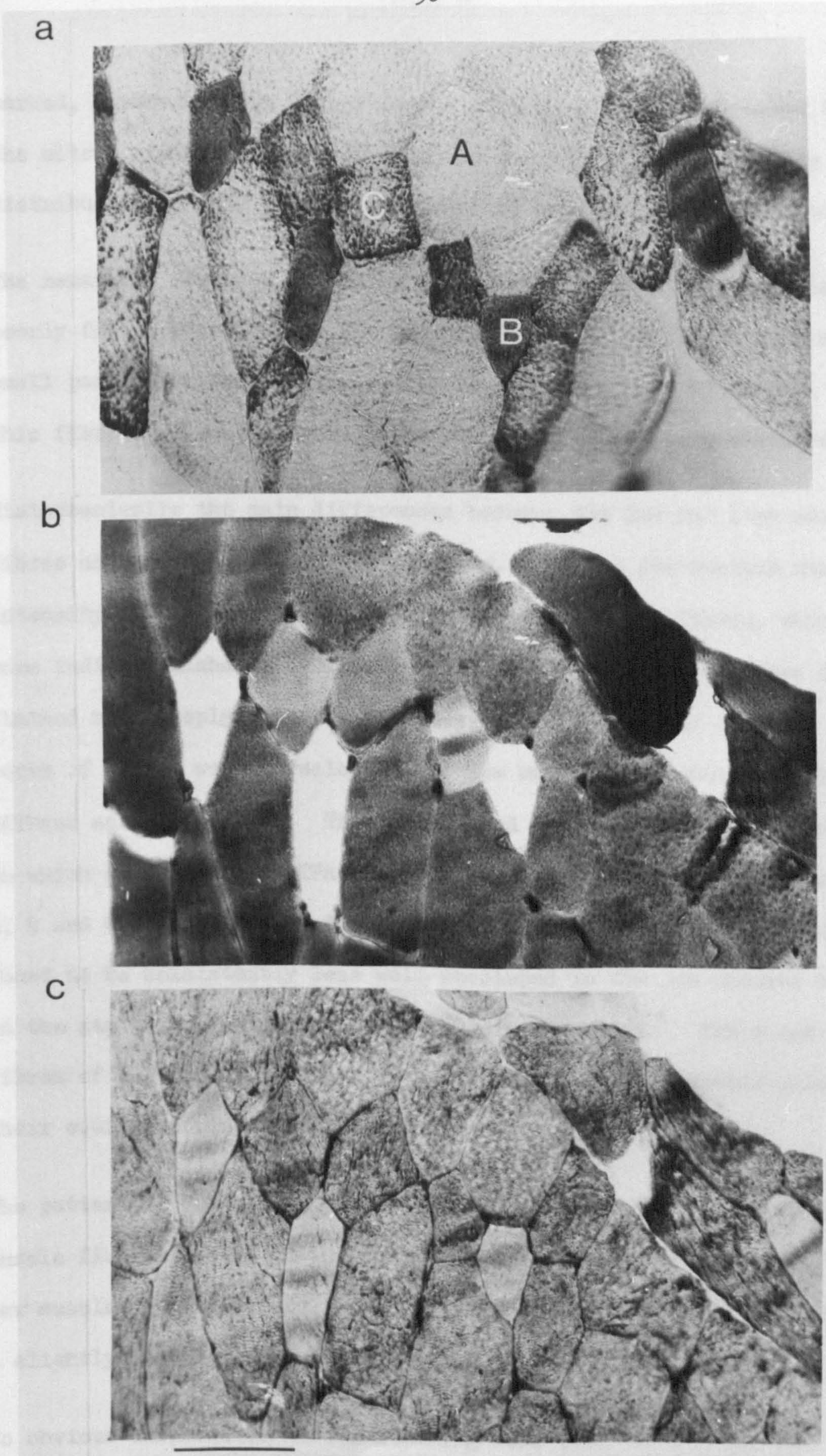

Fig. 1.12

The histochemical profiles of individual fibres identifiable in serial sections of gastrocnemius muscle stained for (a) SDH, (b) MATP'ase and (c) PAS. Calibration bar is 100μm.

marked, although often discontinuous band. Staining indicated that the mitochondrial content was high and largely subsarcolemmal in distribution. This fibre type resembled the C fibres of gastrocnemius.

The remaining minority of fibres had low MATPase activity and stained poorly for glycogen. The SDH reaction was well developed with many small particles being evenly scattered throughout the cytoplasm. This fibre type most closely resembled the B fibres of gastrocnemius.

Histochemically the main differences between the jaw and limb muscle fibres concerned their MATPase staining. In the jaw muscles the intensity of the reaction was similar in the A and C fibres, which were indistinguishable by this technique. Both of these fibre types stained more deeply than the B fibres, and consequently only two sorts of fibres were revealed in the jaw muscles according to their MATPase activity alone. This contrasted with the cat gastrocnemius in which staining for MATPase, at pH 9.4, gave a clear separation of A, B and C fibre types. Also, rather surprisingly, the reaction was found to be consistently less well developed in the jaw muscles than in the gastrocnemius with which they were incubated. The A and C fibres of the former groups invariably proved to be somewhat paler than their equivalents in the limb muscle.

The pattern of SDH activity was almost identical in the jaw and limb muscle fibre types. It was noted, however, that the A fibres of the jaw muscles were less completely free of enzyme reaction, indicating a slightly higher mitochondrial content.

No obvious differences in PAS staining were seen between jaw and limb muscles. In both, the glycogen content of A and C fibres was greater than that of the B type, but systematic classification could not be

made by this method. Neither was Sudan black particularly helpful, for although generally following SDH activity it was never as clear an indicator of mitochondrial distribution.

Table 1.2 summarizes the histochemical features of the three principal jaw muscle fibre types, whilst the cross-sectional areas and relative numbers of individual fibres for which complete histochemical profiles were available are presented in Table 1.3.

A fibres were universally the most common. In the masseter and pterygoid muscles B fibres outnumbered the C type, although this was reversed in the temporalis.

In each jaw muscle the A fibres had a significantly greater cross-sectional area than the C fibres ($p = 0.001$) which in turn were significantly larger than the B fibres ($p = 0.001$).

The results quoted were obtained by combining data from sections taken from all parts of the jaw muscles. No obvious differences either in fibre types or in their relative numbers were noted between the various regions of any of the muscles although this possibility was not studied in detail.

MATPase staining also proved useful as a marker of blood vessels, since capillary walls show high enzyme activity. All of the jaw muscles had an excellent blood supply with each type of fibre commonly surrounded by three or four capillaries. This contrasted with gastrocnemius in which there were fewer blood vessels and a gradation in the number of capillaries associated with particular fibre types. In this muscle most vessels were related to the B fibres and least to the type A.

TABLE 1.2 HISTOCHEMICAL CHARACTERISTICS OF JAW MUSCLE FIBRE TYPES

REACTION	TYPE A	TYPE B	TYPE C
MATP'ase	High activity throughout fibres.	Low activity.	High activity throughout fibres.
S.D.H. (mitochondrial content)	Low activity. Mitochondria sparse, mainly peripheral.	High activity. Mitochondria evenly distributed throughout fibres.	High activity. Mitochondria concentrated peripherally.
P.A.S. (glycogen content)	High	Low	High

Table 1.3

NUMBERS AND SIZES OF FIBER TYPES IN JAW MUSCLES[a]

Muscle	TYPE A %	TYPE A Mean cross-sectional area (μm^2)	TYPE B %	TYPE B Mean cross-sectional area (μm^2)	TYPE C %	TYPE C Mean cross-sectional area (μm^2)
Masseter	82	4100 (SD 1210)	10	1400 (SD 809)	8	2670 (SD 545)
Pterygoid	51	3380 (SD 840)	29	1300 (SD 447)	20	2330 (SD 463)
Temporalis	72	3770 (SD 826)	2	871 (SD 284)	26	2590 (SD 774)

[a] In each muscle estimates were based on 200 fibers for which full histochemical profiles were obtained.

(from Taylor, Cody & Bosley, 1973)

1.5 DISCUSSION

The cat jaw-closing muscles, masseter and temporalis, were found to be very rapidly contracting, confirming the earlier observations of Taylor & Davey (1968). Isometric twitch times were intermediate between those of the extraocular muscles (Cooper & Eccles, 1930; Bach-y-Rita & Ito, 1966) and those of the fastest limb muscles (e.g. FHL, Buller, Eccles & Eccles, 1960).

It is well known that the time course of twitches depends, to some extent, on initial muscle length. In the present experiments speeds were measured at the optimal length for twitch tension (L_{Tw}). For strips in which comparison was made, this length closely corresponded to that for maximal tetanic tension (L_{Tet}), in common with a number of other muscles (e.g. FHL, Buller & Lewis, 1963). However, a frequent finding is that L_{Tw} is 5-10% greater than L_{Tet} (Close, 1972) and it has been shown in this situation, e.g. rat gracilis anterior, that the duration of twitches may increase with length between L_{Tet} and L_{Tw} (Bahler, Fales & Zierler, 1967). This suggests that the present short twitch times cannot have arisen from the initialization of muscle length to an inappropriately low value. Indeed, the use of L_{Tw} is more likely to give a slight underestimate of twitch speed. It is interesting to note that considerable variation between L_{Tw} and L_{Tet} has been found for many individual motor units in cat FHL, although these lengths are similar for the whole muscle (Lewis & Luck, 1968).

The relative speed of the cat jaw muscles fits in well with the general rule that in a given species the contraction time of a muscle is inversely related to its size. The fastest muscles, e.g. extraocular muscles (Tp = 5.7 - 10.0 msec, Cooper & Eccles, 1930; Bach-y-Rita &

Ito, 1966) and thyroarytenoid (Tp = 9.0 - 13.0 msec, Hall-Craggs, 1968) are small, whereas the larger hindlimb muscles are slower, e.g. gastrocnemius (Tp = 22.5 msec, Wills, 1942) and soleus (Tp = 73.0 - 78.0, Gordon & Phillips, 1953; Buller & Lewis, 1965).

In common with other muscles having short twitch times the Tet:Tw ratio was high for the jaw muscles, being almost twice that reported for the limb muscles (Buller & Lewis, 1965).

Although apparent tetanic fusion was obtained at about 100/sec, in the jaw muscles, the rate of rise of tetanic tension continued to increase at higher frequencies of stimulation. The maximal value of $\frac{dP_{tet}}{dt}$ of 3-5% Po/msec and frequency necessary to achieve this were comparable with those of FHL, and greater than the corresponding values for the slow soleus (Buller & Lewis, 1965). In the extraocular muscles still higher frequencies are reported to produce maximal $\frac{dP_{tet}}{dt}$ and a significant increase in this parameter is seen between 400 and 600 i.p.s. (Barmack, Bell & Rence, 1971; Fuchs & Luschei, 1971). Post-tetanic potentiation was not great in the jaw muscles, never exceeding 130-140%. This value is lower than that observed in many fast muscles, in which post-tetanic twitch tension may be doubled, but is greater than that seen in slow muscles in which no change or a slight reduction, i.e. post-tetanic depression, is commonly found (see Close, 1972).

The decline in tetanic tension during repetitive trains of stimuli took place in two main phases. In the early part of fatigue tension fell rapidly to a value which thereafter showed little further reduction. The simplest interpretation of these observations is that the two phases of fatigue resulted from two functionally distinct groups of motor units.

In this way the initial fall in tension would be accounted for by the rapid fatigue of a group of large motor units whilst the subsequent period of maintained force would result from the remaining group of small units.

Three principal histochemical fibre types were found in the cat jaw muscles, as has been described in the mixed hindlimb muscles. Almost all fibres could be confidently allocated to one of three groups according to a combination of their density of MATPase staining and distribution of SDH activity. However, variation was seen within each fibre type, especially regarding SDH staining. This was particularly true of C fibres, which although all characterized by preferentially peripheral SDH activity, showed a range of staining within the more central part of the fibres.

The most obvious difference in the histochemistry of the cat jaw and mixed hindlimb muscles was that in the former the intensity of MATPase staining of A and C fibres was similar and that together these two fibre types constituted a far higher percentage of the cross-sectional area. In each of the jaw muscles at least 95% of the cross-sectional area was composed of fibres of relatively high MATPase activity.

This finding, in the light of the short twitch times of the jaw muscles, appears to be consistent with the hypothesis that MATPase is important in determining contraction speed (see Close, 1972). However this supposition is complicated by the observation that in the jaw muscles, stained at pH 9.4 to demonstrate this enzyme, the intensity of the reaction of A and C fibres was always less than that of their equivalents in simultaneously prepared gastrocnemius. Physiological recordings show that twitch speeds of FF and FR motor units, believed to correspond

to respectively A and C fibres, are less than those of the jaw muscles despite higher MATPase activity (Burke, Levine, Tsairis & Zajac, 1973).

A possible explanation is that the MATPases of fast fibres in jaw and limb muscles are chemically distinct proteins. Certainly there appear to be dissimilarities in the chemical structures of fast and slow myosins, particularly regarding their 3-methylhistidine content (see Close, 1972). Also Guth, Samaha & Albers (1970) have demonstrated histochemically that MATPases of various fibre types differ in pH stability. Whether comparable differences exist between different muscles of the same species has not been assessed. However, it seems possible that the unexpectedly low MATPase staining of the jaw muscle fibres, compared to those of the limb muscles, could have arisen from differences in the alkali stability of the enzyme. It should be remembered that the histochemical demonstration of Ca^{++} activated MATPase is conventionally done at the artificial pH of 9.4. Under these conditions solubilization of the reaction product is prevented and also fibre types may be most clearly distinguished according to the intensity of their reaction. At physiological pH the method does not work well. Since it is difficult to interpret the relationship between enzyme activity demonstrated at this pH and that in vivo it is unwise to attempt quantitative comparisons from histochemical techniques.

Correlation of contraction speed and MATPase activity is to be expected if the maximal rate at which actin and myosin filaments slide past each other during contraction depends largely on the rate of hydrolysis of ATP. This is not to say that MATPase activity is the rate-limiting factor determining contraction speed, especially in very fast muscles, since other factors also regulate the properties of contractile proteins.

The kinetics of excitation-contraction coupling seem certain to play a part in defining the time course of rapid twitches. In particular the duration of the active state is widely thought to be related to the exchange of Ca^{++} ions between the sarcoplasm and sarcoplasmic reticulum (SR). During relaxation the interaction of myosin and actin filaments is thought to be inhibited by the troponin-tropomyosin complex on the contractile material. Excitation causes the release of Ca^{++} ions from the SR into the sarcoplasm, the binding of these ions to troponin and the removal of the inhibitory effects of the complex. Conversely, the return to the resting state involves the separation of Ca^{++} ions from the troponin-tropomyosin complex and their subsequent sequestering by the SR (Sandow, 1970). Kinetic studies of SR, in vitro, show that the rate of Ca^{++} ion uptake by fast muscle fragments is far greater than for slow muscles (Fiehn & Peter, 1971). Also the SR of fast muscles appears to be more extensive (Fiehn & Peter, 1971; Harigaya, Ogawa & Sugita, 1968). In this context a study of the SR of very rapidly contracting muscles would be interesting.

In studies comparing the dynamic properties of the fast rat inferior rectus muscles and the slower extensor digitorum longus, Close (1974) found that differences in isometric twitch times could not be attributed to differences in the intrinsic speed of shortening of sarcomeres. Since the relationship between intrinsic speed of shortening and relative load was approximately the same for these muscles he concluded that differences must exist in the relationship between intrinsic speed of shortening of contractile material and the duration of the active state.

It has been previously suggested that the behaviour of the jaw muscles during repetitive stimulation could be best accounted for by the presence of two groups of motor units, respectively rapidly and slowly fatiguing.

The division of fibres into those poor (type A) and those rich (types B and C) in mitochondria is broadly that anticipated from the theory that mitochondrial content determines fatiguability (Henneman & Olson, 1965). The large initial fall in tetanic tension would be attributed to rapid fatigue of the predominant A fibres, whilst more gradual fatigue of B and C fibres could account for the second phase. The presence of many mitochondria, with high oxidative enzyme activity, but little glycogen in B fibres suggests that aerobic mechanisms may be largely responsible for the supply of energy. Conversely the high glycogen content and relative absence of mitochondria in A fibres is consistent with these depending largely on anaerobic processes. Presumably both glycogenolysis and oxidative pathways are important in C fibres.

These interpretations are consistent with findings in single motor unit studies in cat gastrocnemius muscles, in which FF units showed early fatigue during repetitive trains of stimuli, whilst the tetanic tension of FR units was only slightly reduced over the first 6 min of stimulation and that of S units was unchanged for up to 1 hr (Burke, Levine, Zajac, Tsairis & Engel, 1971).

Two stages of fatigue have been described in certain human muscles, e.g. first dorsal interosseous (Stephens & Taylor, 1972). In this study early fatigue, during maintained maximal voluntary contraction, was thought to be due to units in which neuromuscular junction (NMJ) fatigue predominated, whereas later, presumably in a different group of units, the contractile elements themselves appeared to be the principal site.

In the absence of simultaneously recorded EMG the mechanisms responsible for fatigue in the jaw muscles are uncertain. In the cat gastrocnemius Burke, Levine, Tsairis & Zajac (1973) found no reduction in motor unit action potential amplitude during repetitive trains of 40 stimuli/sec and concluded that fatigue in each of the three groups of units occurred in the contractile machinery. However, in a more recent study (Stephens & Stuart; personal communication), under similar experimental conditions, a reduction in electrical activity was commonly seen. Consequently the possibility of NMJ fatigue cannot be ruled out in the present experiments in which higher stimulation frequencies (60-90/sec) were used.

As previously pointed out C fibres of the cat jaw muscles showed a range of SDH staining. Burke and coworkers (1973) have suggested that, in the cat gastrocnemius, FF (corresponding to A fibres) and FR (C fibres) motor units may be interconvertible. In addition, the plastic nature of histochemical fibre types following exercise and cross-innervation has long been recognized (see Close, 1972). Such alterations, according to usage, could acccount for differences in the findings of Burke and his collaborators (1973) and Stephens & Stuart (personal communication) concerning motor unit typing. Whereas the former group claimed that motor units could, almost without exception, be divided into three categories, the latter workers found a substantial proportion of units with intermediate properties. This suggests that a more flexible approach to motor unit classification may prove most satisfactory.

1.6 SUMMARY

1. Strips of the jaw-closing muscles, masseter and temporalis, were fast with mean Tps of 13.1 msec and 11.4 msec respectively during isometric contractions. Corresponding $T_{\frac{1}{2}R}$ values were 12.8 msec and 9.81 msec.

2. Tetanic fusion frequency was about 100/sec and the maximal rate of rise of tetanic tension of 4-5% P_o/msec was at 400-500/sec.

3. $Tet/_{Tw}$ ratios were high.

4. PTP was typically 130-140% of the pretetanic value.

5. Fatigue occurred in two main phases during trains of repetitive stimuli at 60-90/sec. Over the first minute tetanic tension fell to about one-quarter of its initial value. Thereafter only a gradual decline in tension was seen.

6. Three main histochemical fibre types were found, corresponding to A, B and C fibres in cat mixed hindlimb muscle. Jaw muscle fibre types differed mainly in their MATPase staining, A and C fibres showing similar intensities of reaction. All three jaw muscles had a far greater proportion of their cross-sectional area composed of fibres with relatively high MATPase activity.

SECTION 2

FUNCTIONAL CELL TYPES PRESENT IN THE MeNV

2.1 INTRODUCTION

The MeNV consists of sparsely scattered collections of cells situated bilaterally between the periaqueductal grey matter and the mesencephalic reticular formation, and extends from the level of the posterior commissure to that of the trigeminal motor nucleus (Figs. 2.1, 2.2). Morphologically the constituent cells closely resemble dorsal root ganglion cells, being large, oval and predominantly unipolar.

In recent years it has been generally accepted that these cells are first order sensory neurones, but there has been considerable disagreement as to their peripheral origin.

Four principal functional cell types have been claimed to be present in the MeNV, namely somata of (1) muscle spindle afferents of ipsilateral jaw-closing muscles (masseter, medial pterygoid and temporalis), (2) tendon organ afferents of ipsilateral jaw-closing muscles, (3) ipsilateral dental mechanoreceptor afferents and (4) ipsilateral extraocular muscle stretch receptor afferents.

An interesting feature of the MeNV, recently demonstrated in the rat, is electrotonic coupling between certain neurones (Llinas & Baker, 1971). A conspicuous feature of the nucleus in several species is grouping of cells, particularly in the caudal region, and Hinrichsen & Larramendi (1968) have described soma-somatic and

Fig. 2.1

Sagittal section (2.3 mm lateral to midline) of the cat brainstem stained by the Nissl method (from Berman, 1968). The rostro-caudal extent of the mesencephalic nucleus of the trigeminal nerve (5ME) is indicated by the arrows. Horsely-Clarke coordinates are shown in mm.

SCS, SCI and SCD : Layers of Superior Colliculus.
ICP, ICC : Layers of Inferior Colliculus.
PAG : Periaqueductal grey matter.
CB : Cerebellum.
V4 : Fourth ventricle.
4N : Fourth cranial nerve.

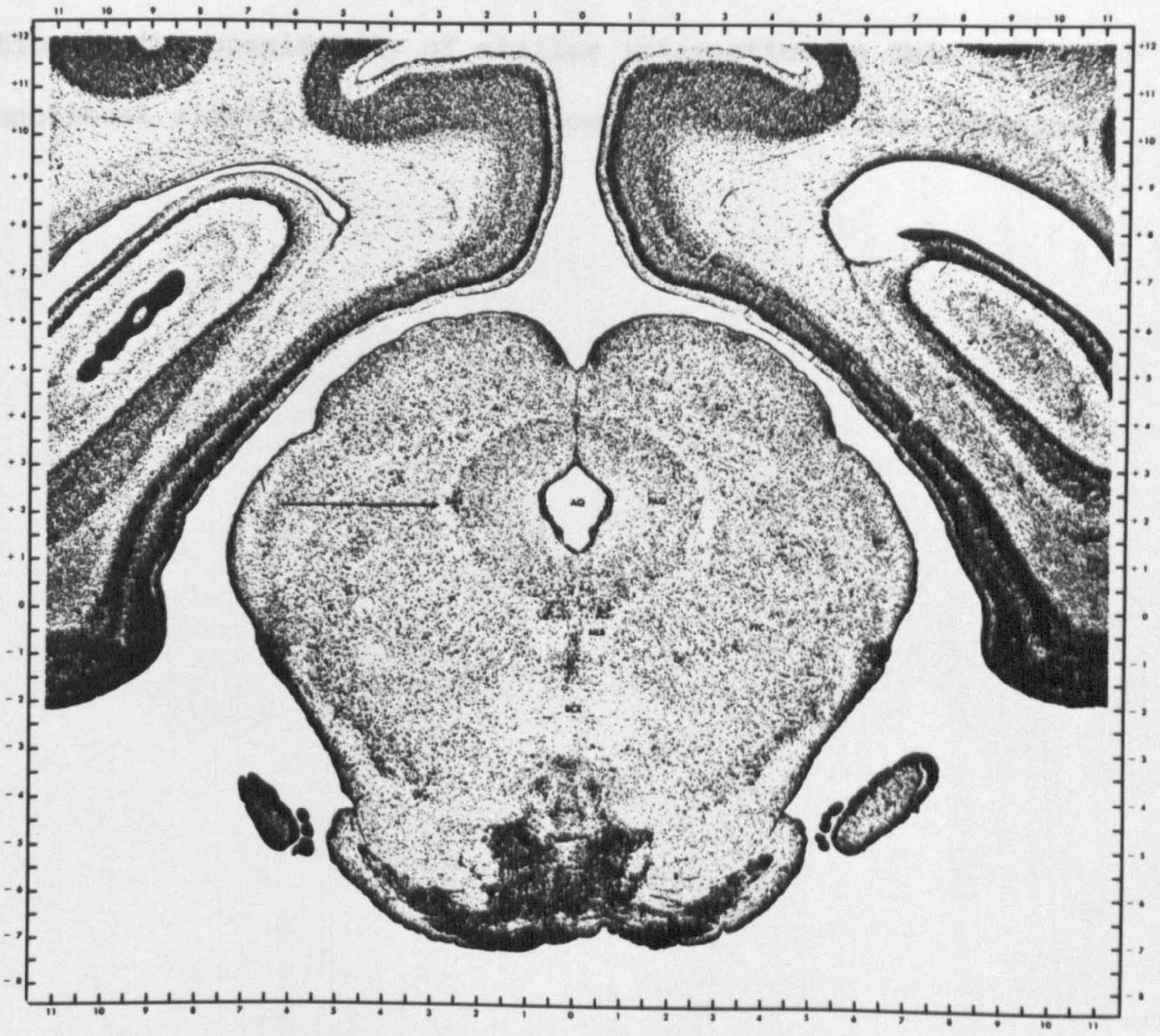

Fig. 2.2

Frontal section (1.6 mm rostral to interaural line) of the cat brainstem stained by the Nissl method (from Berman, 1968). Cells of the 5ME are located at the edge of the periaqueductal grey matter as indicated by the arrow. Horsely-Clarke coordinates are indicated in mm. Abbreviations as for Fig. 2.1.

soma-axonal connexions in electron microscopic studies in the mouse.

Fig. 2.3 illustrates grouping of cells in the cat MeNV and draws attention to the possibility of similar interaction in this species. The functional significance of such coupling has not been explained.

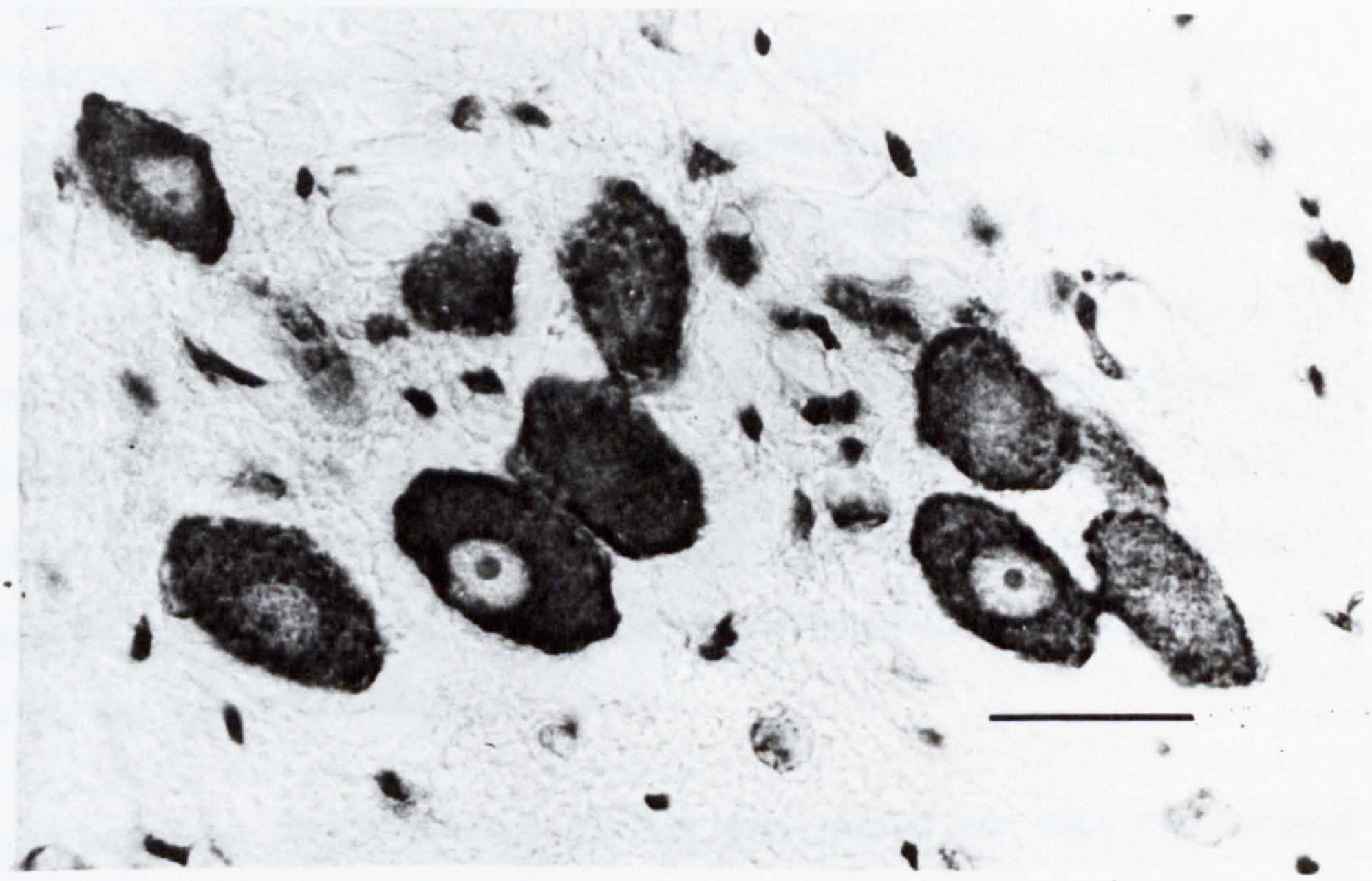

Fig. 2.3 Cell grouping in the Mesencephalic Nucleus. Calibration bar represents 50μ.

2.2 HISTORICAL REVIEW

2.2.1 Evidence from Histological Studies

The MeNV was initially believed to be motor in function. Early workers regarded the nucleus as the origin of the trochlear nerve (Stilling, 1846; Deiters, 1865; Golgi, 1894). Later Meynert (1867) demonstrated that the mesencephalic root was a component of the fifth cranial nerve, rather than the fourth, and that its fibres arose from the MeNV. However, whilst agreeing with this finding, many authors still accepted the centrifugal nature of the fibres (Henle, 1879; Held, 1893; Kollicker, 1896; Cajal, 1909). This belief was based largely on the passage of fibres from the nucleus through the portio minor of the trigeminal nerve, which was known to be the motor division.

An alternative theory current during this period was that the nucleus was associated with autonomic functions (Huguenin, 1873).

Meynert (1867), in addition to being the first clearly to classify the MeNV as belonging to the fifth cranial nerve, also suggested a possible sensory role, together with several others (Krause, 1876; Bechterew, 1899). Johnston (1909) did much to establish this fact by a series of histological and embryological studies, in a range of vertebrates. He stressed the similarity of the morphological features of the MeNV and those of dorsal root ganglion cells and reinforced this by demonstrating that the embryonic origin of these cells was the dorsal alar plate as for other sensory cells.

Thus Johnston recognised the MeNV as a unique example of a sensory ganglion that during evolution had migrated within the neuraxis. Subsequent authors in a variety of histological and degeneration experiments were led to a similar conclusion (Willems, 1911; Kosaka, 1912; Clark, 1926; Weinberg, 1928; Corbin, 1940; Szentagothai, 1948).

Whilst the earliest studies were mainly concerned with determining the intracerebral course of MeNV fibres, after the turn of the century more attention was directed towards their peripheral distribution. An association between the nucleus and the masticatory muscles was soon established. Willems (1911), working in the rabbit, observed chromatolysis of MeNV cells after severing individual nerves to the jaw muscles and in the following year Kosaka (1912) obtained similar results upon cutting the mandibular nerve. These findings were supported by experiments in the guinea-pig (Allen, 1919) and cat (Thelander, 1924; Corbin, 1940) by the production of degeneration of fibres in the masseteric, pterygoid and temporal nerves by section of the mesencephalic root. Szentagothai (1948) showed that the muscle receptors concerned were probably spindles when he obtained fragmentation of both annulospiral and flowerspray endings of cat masticatory muscles following midbrain lesions of the mesencephalic tract. The same lesions did not produce changes in tendon organ afferent endings in these muscles and motor fibres appeared intact.

Histological evidence concerning the projection of sensory fibres, other than those from the jaw muscle proprioceptors, to the MeNV,

is less clear. Terterenjanz (1899), who believed the nucleus to be motor, claimed that removal of palatine muscles, in the cat, resulted in degeneration of MeNV tract fibres. These findings were contradicted by Kure (1899) who saw no such changes when repeating this experiment in the dog.

However, the possibility of fibres from the extraocular muscles terminating in the MeNV has received more support. As previously mentioned, several early investigators (e.g. Stilling, 1846; Golgi, 1894) thought that MeNV fibres ran in the trochlear nerve. Freeman (1925) and Sheinin (1928) found chromatolysis of cells in the nucleus and degeneration of fibres of the third, fourth and sixth nerves after removal of the orbital contents in cats and Woollard (1931) reported similar changes in the nucleus after cutting the third nerve in the rabbit. Tarkhan (1934) traced MeNV fibres to the third, fourth and sixth nerves in silver stained sections of cat brain.

On the other hand, Kohnstaumm & Quensel (1908) obtained no signs of MeNV degeneration after destruction of the orbital contents. Also May & Horsley (1910) found that peripheral nerves to the extraocular muscles were unaffected by section of the mesencephalic tract and Tozer (1912) elicited no MeNV damage after severing the third nerve.

In perhaps the most extensive study to date Corbin (1940) lesioned areas of the MeNV and examined all of the cranial nerves after a suitable period. Degeneration was limited to the fifth nerve, being present in the ipsilateral ethmoidal branches of the ophthalmic nerve, the palatine and superior alveolar branches of the maxillary

nerve, and inferior alveolar and masseteric, pterygoid and temporal branches of the mandibular nerve. This indicates that only fibres from the masticatory muscles, palate, gingiva and periodontal membranes project to the MeNV.

2.2.2 Evidence from Electrophysiological Studies

In the first electrophysiological investigation of the MeNV, Corbin & Harrison (1940) recorded multiunit activity in response to stretching ipsilateral masticatory muscles and to pressure on the ipsilateral teeth and hard palate. The discharges from these dental units closely resembled those previously described by Pfaffman (1939) in peripheral branches of the fifth nerve and were believed to arise from receptors in the periodontal membranes. Latency studies on a limited number of such units led Corbin & Harrison (1940) to accept these cells as first order.

Subsequently Cooper, Daniel & Whitteridge (1953) in the goat, and Fillenz (1955) in the cat, made passing comment on the presence of a jaw muscle proprioceptor projection to the MeNV. Similar findings were also obtained in the dog (Kawamura, Funkakoshi & Tsukamoto, 1958).

In a more detailed study in the cat Jerge (1963) showed that the muscle receptors concerned were probably exclusively spindles, by their responses to twitch contractions, and that these were located in the ipsilateral masseter, medial pterygoid and temporalis muscles. He also confirmed the presence of dental mechanoreceptors and classified these according to whether they innervated one or more teeth. No evidence was found of somatotopic organisation within the nucleus. Muscle spindle afferent cells were located throughout the rostro-caudal extent of the nucleus with no apparent segregation according to muscle of origin. Dental receptors were more common caudally. Taylor & Davey (1968) also identified jaw-closing muscle spindle units and dental mechanoreceptor afferent units. The

proprioceptor afferents in addition to having spindle-like responses to passive stretch were activated by suxamethonium (SCh).

The comparative physiology of the MeNV has been studied in birds (Manni, Bortolami & Azzena, 1965; Bortolami & Veggetti, 1967) and reptiles (Desole, Palmieri & Veggetti, 1970) as well as in mammals. In both of these classes "short latency" spindle-like responses were obtained from wide areas of the midbrain upon stretching the jaw muscles.

Smith, Marcarian & Niemer (1967), recording multiunit evoked potentials simultaneously from the right and left MeNV of the cat and monkey, claimed to have obtained bilateral responses, presumably from jaw muscle proprioceptors, upon manipulation of one muscle. These workers attributed contralateral responses to cells with bi- or polysynaptic connexions.

Subsequently, Smith (1969) reported the presence of Golgi tendon organ afferents in the MeNV, based on the finding that some units showed increased firing during twitches of the masseter muscle.

The theory that ocular proprioceptive fibres project to the MeNV has also received some electrophysiological support. Cooper, Daniel & Whitteridge (1953) recorded "low threshold, short latency" responses to stretching ipsilateral extraocular muscles from a widespread area of the brainstem of the goat, including the MeNV. Later Fillenz (1955) obtained comparable early sustained responses along the course of the nucleus in the cat. In contrast Corbin & Harrison (1942) could only locate cells that resembled extraocular sensory afferents in the areas of the ocular and trochlear motor

nuclei, while evidence in the sheep and pig (Manni, Bortolami & Desole, 1966, 1968) points to the semilunar ganglion as the site of such neurones.

2.3 METHODS

Forty adult cats, male and female, in the weight range 2-3 kg, were used in this section of the work.

The general experimental set up is shown in Fig. 2.4.

2.3.1 Anaesthesia

Animals were anaesthetized with sodium pentobarbitone (60 mg/kg, I.P.) and subsequently maintained at the required level by small dilute intravenous supplements via a forelimb vessel. Cats were generally kept deeply anaesthetized as judged by the absence of withdrawal, pupillary and pinna reflexes. This was because the neurones of interest were first order and it was desired to study these as free as possible from other neural influences. Barbiturates are known to produce widespread depression within the CNS mainly by their action at synapses in reducing the release of transmitter substances (Løyning, Oshima & Yokota, 1964; Richards, 1972). Consequently bi- and polysynaptic pathways are especially susceptible. In later experiments, while recording from muscle spindle afferents, the tranquilizer chlorpromazine (I.V. doses of 5 mg, half-hourly) was used, in addition, to suppress fusimotor activity. Such doses of chlorpromazine are greater than those shown by Henatsch & Ingvar (1956) to abolish spontaneously and reflexly evoked fusimotor discharge, particularly in combination with pentobarbitone which has similar, although less specific, actions (Voorhoeve & van Kanten, 1962). The fusimotor pathway was removed pharmacologically rather than surgically because peripherally the muscle spindle afferents run in the motor division of the tri-

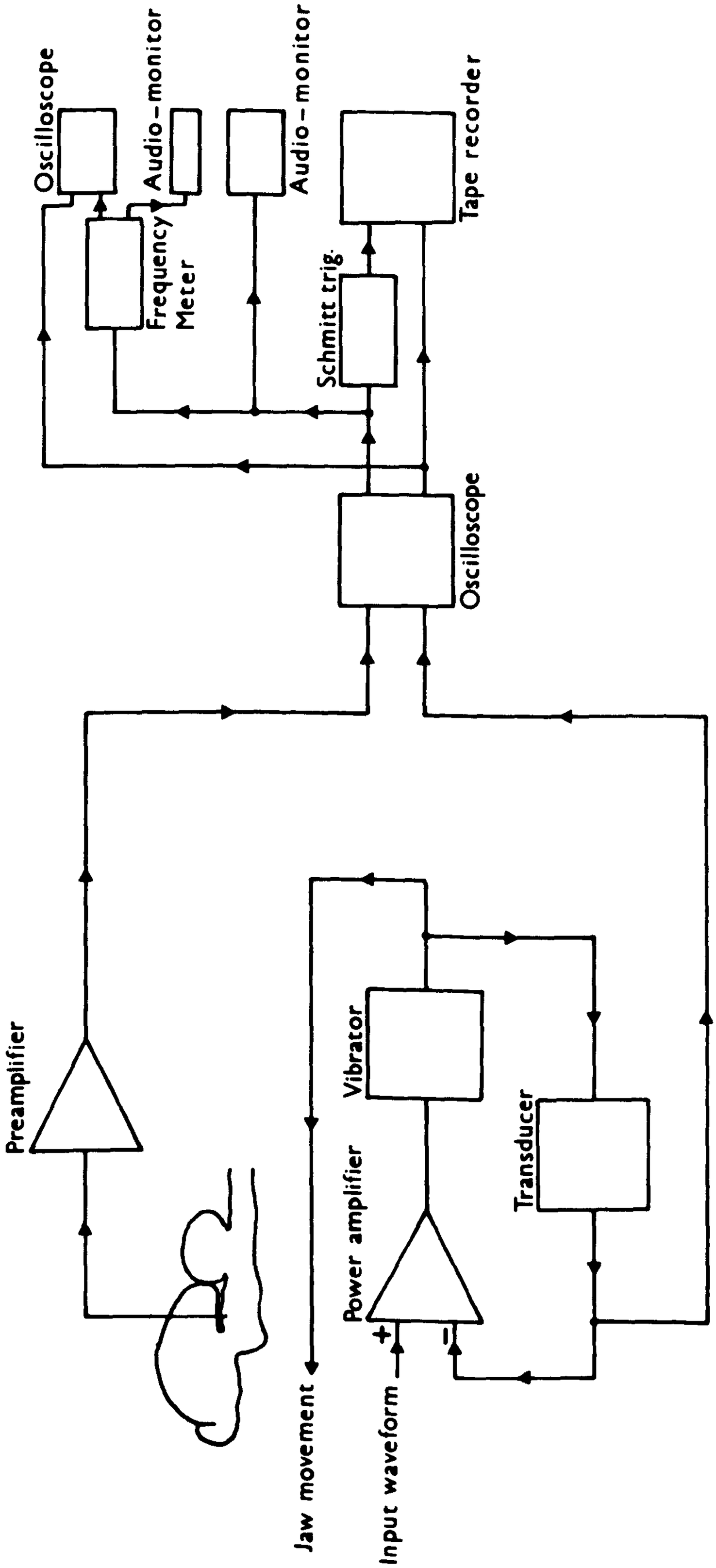

Fig. 2.4

General experimental set-up.

geminal nerve and, centrally, ablation of the trigeminal motor nucleus would involve a serious risk of damaging the caudal part of the MeNV.

2.3.2 Operative Procedures

The trachea was routinely intubated to ensure a clear airway and to permit artificial ventilation in some experiments. A forelimb vein was cannulated for the administration of anaesthetic and of other drugs. Occasionally one common carotid artery was also cannulated towards the head. Body temperature was maintained at 37-38^{o}C by a homeothermic blanket (Epil, 240H).

The animal's head was mounted in a stereotaxic device (La Précision Cinématographique, for visual experiments) as shown in Fig. 2.5. A midline incision was made and one temporalis muscle reflected a short way. The cortex overlying the midbrain was revealed by burring a small hole, unilaterally, in the skull which was then enlarged with bone cutters, until it extended some 1.5 cm from the midline over a length of 3-4 cm. In many animals the superior colliculus was exposed by hemispherectomy, and in all experiments in which muscle relaxants were used decerebration was carried out. Access to the more caudal regions of the MeNV was provided by drilling away part of the bony tentorium cerebelli.

2.3.3 Electrical Recording from the MeNV

The MeNV was normally reached by inserting electrodes vertically through the hole in the skull according to stereotaxic co-ordinates calculated from an atlas of the cat brainstem (Berman, 1968). Glass-coated tungsten microelectrodes (Merrill & Ainsworth, 1972)

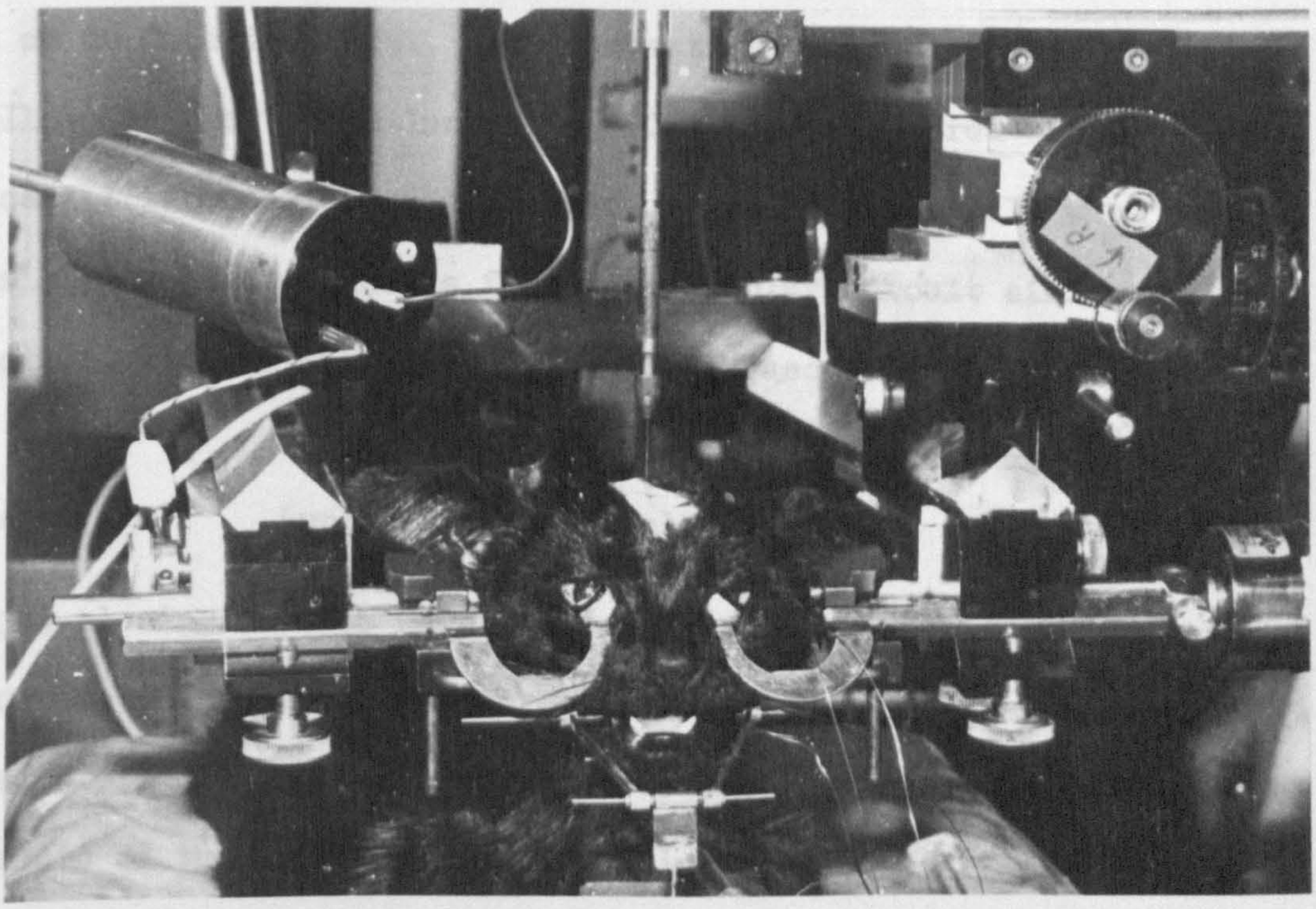

Fig. 2.5

The animal's head is secured in a stereotaxic device . Recordings from the MeNV are made using glass-coated tungsten microelectrodes, advanced by a micrometer drive. The electrode is initially connected to a cathode follower, prior to further amplification of electrical signals. Jaw muscle stretch is applied by a vibrator via a metal frame attached to the mandible. Displacement of the jaw is measured by an instrument potentiometer.

were used, with impedances 1-3 MΩ at 1.7 kHz. Signals were amplified by a cathode follower (400 MΩ input impedance at 1 kHz) and a preamplifier (Tektronix Inc., type RM 122) with 3 dB frequency cuts at 80 Hz and 10 kHz. Nervous activity was displayed on an oscilloscope (Tektronix Inc., type 502A) and monitored with an audioamplifier and loudspeaker unit. Action potentials were used to trigger an instantaneous frequency display circuit similar, in principle, to that described by Huxley & Pascoe (1963).

2.3.4 Application of Muscle Stretch

The jaw closing muscles were stretched by passive jaw opening movements. In the cat the three principal jaw closers, masseter, medial pterygoid and temporalis act to produce simple hinge-like movements of the temporomandibular joint (TMJ), with little lateral deflection. The mandible was secured to a light-weight V-shaped metal frame (Fig. 2.6), pivoted about an axis passing through the TMJs. The apex of the V was coupled to an electromagnetic displacement servo (Pye-Ling V50 vibrator). Sinusoidal and ramp waveforms were used to drive the vibrator by means of a power amplifier (Aim Electronics Ltd., type WPA 116). Displacement feedback was provided by a transducer consisting of a transparency, bearing saw-tooth opacities, mounted between a light source and a photoelectric transistor (Mullard Ltd., type BPX 25). The transparency was placed in the same plane as the shaft of the vibrator so that the intensity of transmitted light activating the phototransistor was proportional to the movement of the shaft and transparency. The output of the phototransistor was then amplified, fed back to the power amplifier and monitored on an oscilloscope (Tektronix Inc., type 502A).

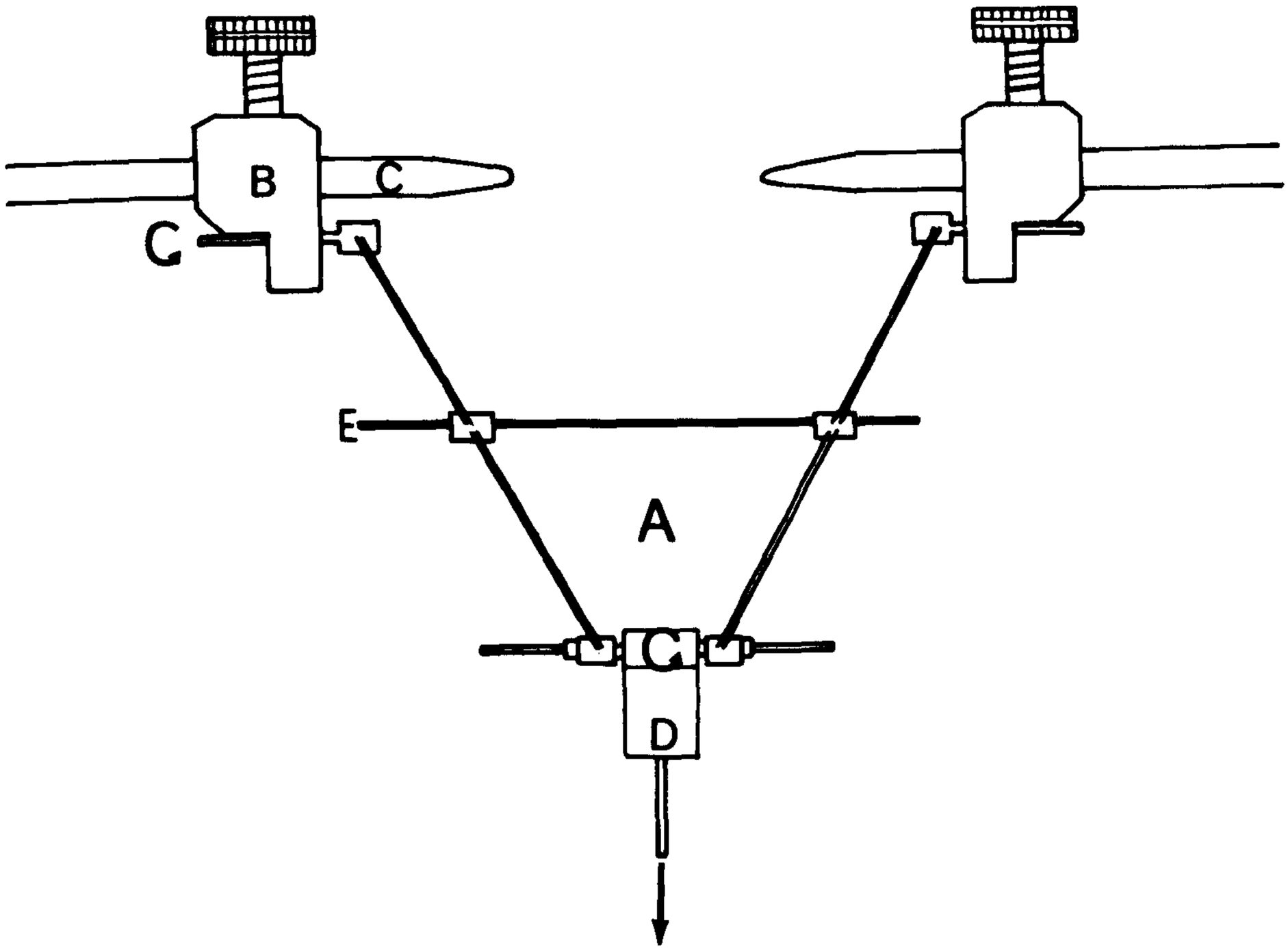

Fig. 2.6

The V-shaped metal frame (A) rotates within holes drilled in the brass blocks (B) attached to the ear bars (C). Thus the frame pivots about an axis closely approximating to that passing through the TMJs. The apex of the V is coupled to the moving part of a vibrator by means of a rotating arm (D). The pin (E) is either firmly tied to the mandible or passed through holes drilled in the lower canines.

Input waveforms to drive the vibrator were obtained from a low frequency oscillator (Dawes, type 445A). Sine waves were either used directly or to generate ramps of variable amplitude and velocity (Fig. 2.7).

Angular displacement of the jaw was measured by means of a light instrument potentiometer whose moving part was attached to the rod about which the jaw pivoted. This rod was orientated in the axis passing through the TMJs and rotated with jaw movements. The output of the potentiometer was displayed on a digital voltmeter (Advance Instruments Ltd., DMM1).

2.3.5 Electrical Stimulation of the Jaw Muscles

Pairs of fine enamelled silver wires (Johnson Matthey Ltd.) with their final 2 mm bared were inserted into each of the jaw-closing muscles using hypodermic needles. The masseter and temporalis were reached through the skin and the medial pterygoid through the palate. Wires were connected to isolated stimulators (Devices Ltd., Mk IV) which controlled the amplitude and duration of pulses. The frequency and length of pulse trains were determined by triggering the isolated stimulators from either a gated pulse generator (Devices Ltd., GPG) or a digitimer (Devices Ltd.).

2.3.6 Tooth Stimulation

Pressure stimuli were applied to teeth using the Pye-Ling vibrator. An extension of the moving arm was placed in contact with the tooth surface and aligned so that its plane of movement corresponded to the direction of maximal sensitivity of the receptor.

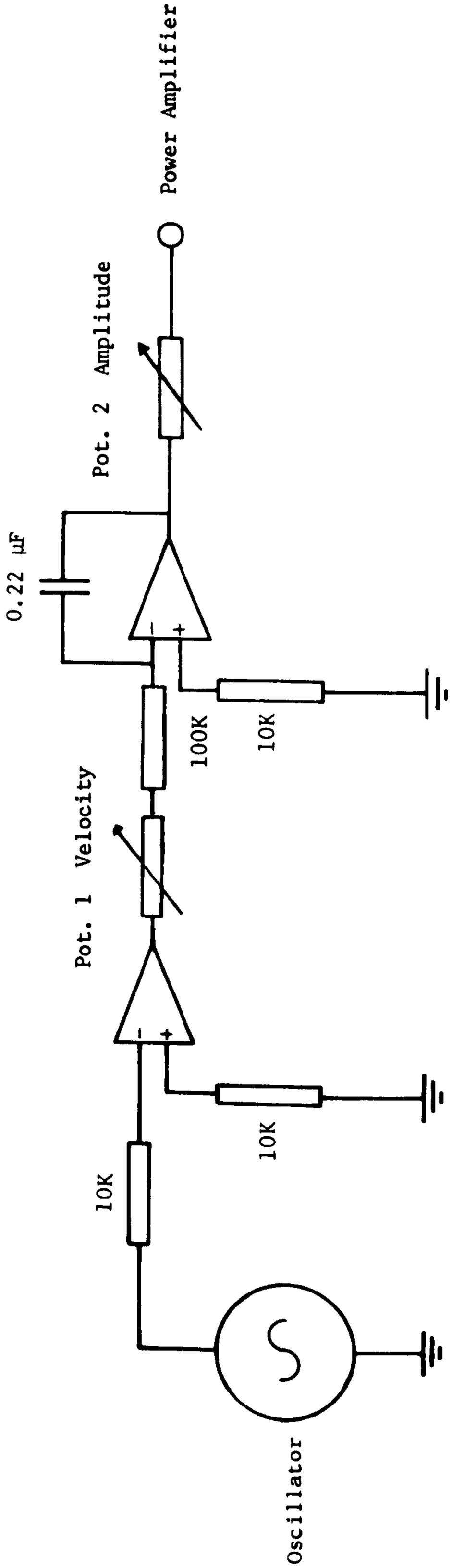

Fig. 2.7 RAMP CIRCUIT

2.3.7 Recording Instrumentation

The outputs of the display oscilloscope vertical deflexion amplifiers were recorded on a 6-channel analogue taperecorder (Levers-Rich, Series E). Nerve action potentials were recorded on a direct recording channel (bandwidth 50 Hz - 25 kHz ± 2 dB) while jaw displacement was recorded on a F-M channel (bandwidth DC to 1200 Hz). Experimental protocol was recorded on a voice channel to facilitate later recognition of taped results.

2.3.8 Photography

The frequency meter output and stretching waveform were displayed on a slave oscilloscope for photography, using a continuous recording camera (Nihon Kohden Kogyo Co. Ltd., type PC-2A) with 35 mm recording paper (Kodak Ltd., RP.30).

2.3.9 Histology

At the end of each experiment the cat was killed by an intravenous pentobarbitone overdose. Brains were fixed in situ, by perfusion, normally through the thoracic aorta, with 10% formol saline at 100 mm Hg, after residual blood had been washed out with isotonic saline.

After decapitation heads were stored in formol saline for a further 4-5 days. Then they were returned to the stereotaxic apparatus and the midbrain sectioned into blocks which were removed. Blocks were frozen using a thermoelectric cooling device (Pelcool Ltd.) and serial coronal sections cut at 10μ with a freezing microtome (Leitz Ltd.). Sections were floated out into water baths and mounted on glycerinated slides. Exposure of these slides

to formalin ensured good adhesion. Sections were then stained with cresyl fast violet.

Midbrains treated in this way were examined for electrode tracks.

2.4 RESULTS

During the exploration of the midbrain, ramp stretches were continuously applied to the jaw with a cycle period of 2 sec. When the region of the dorso-lateral aspect of the central grey matter was approached unitary activity was detected in time with jaw movements. Thereafter, as the electrode was advanced to isolate single units, tests for tooth receptors, and eye and jaw muscle proprioceptors were frequently applied, together with occasional testing for other sensory input from the head. The only activity encountered in this region was related to jaw opening or to tooth pressure.

2.4.1 Dental Mechanoreceptors

Systematic testing for dental receptors was carried out in a preliminary series of six cats. In subsequent experiments no further attention was directed towards these cells.

Eighteen units were identified as arising from dental mechanoreceptors by their responses to pressure on teeth or surrounding gingivae. The mandible was always held rigidly during the application of such stimuli.

Sixteen of these units were activated by pressure to individual ipsilateral teeth, being most commonly associated with maxillary or mandibular canines. A further unit was excited by pressure on both ipsilateral maxillary incisors whilst the receptive field of the remaining unit was wider, including the gingivae and upper lip. Table 2.1 summarizes the proportions of units activated from different teeth. No spontaneously active units were found.

Dental units generally proved to be quite specific for the direction of the stimulus although this varied considerably between individual teeth. The majority of units, especially those supplying single teeth, were exquisitly sensitive and had a well defined threshold (Fig. 2.8). A sharp tap, in the preferential direction would elicit either a single spike or a short burst with a latency of approximately 3-5 msec (Figs. 2.8, 2.9). As stimulus amplitude was increased the latency was often slightly reduced (Figs. 2.8, 2.9).

A range of adaptation was found. Most units were slowly adapting. After an initial fall in discharge frequency, following the application of pressure, a steady train of impulses, at frequencies 50-120 i.p.s. continued for the duration of the stimulus (Figs. 2.8, 2.9). Occasionally very rapidly adapting units were encountered which discharged only a few spikes.

Dental receptors were far less plentiful than jaw opening units but showed no signs of being specifically segregated from them. Indeed both jaw opening units and dental units could often be recorded simultaneously from a given electrode position (Fig. 2.10). However, the dental units were found exclusively in the caudal region of the nucleus.

Fig. 2.11 indicates the rostro-caudal distribution of dental receptor afferent cells in the MeNV in addition to the number of jaw-opening units encountered in the same electrode tracks as a measure of the relative occurence of these two types of cell. In the limited sample of dental mechanoreceptors studied no evidence was found of organisation within the MeNV according to tooth of origin (Fig. 2.12).

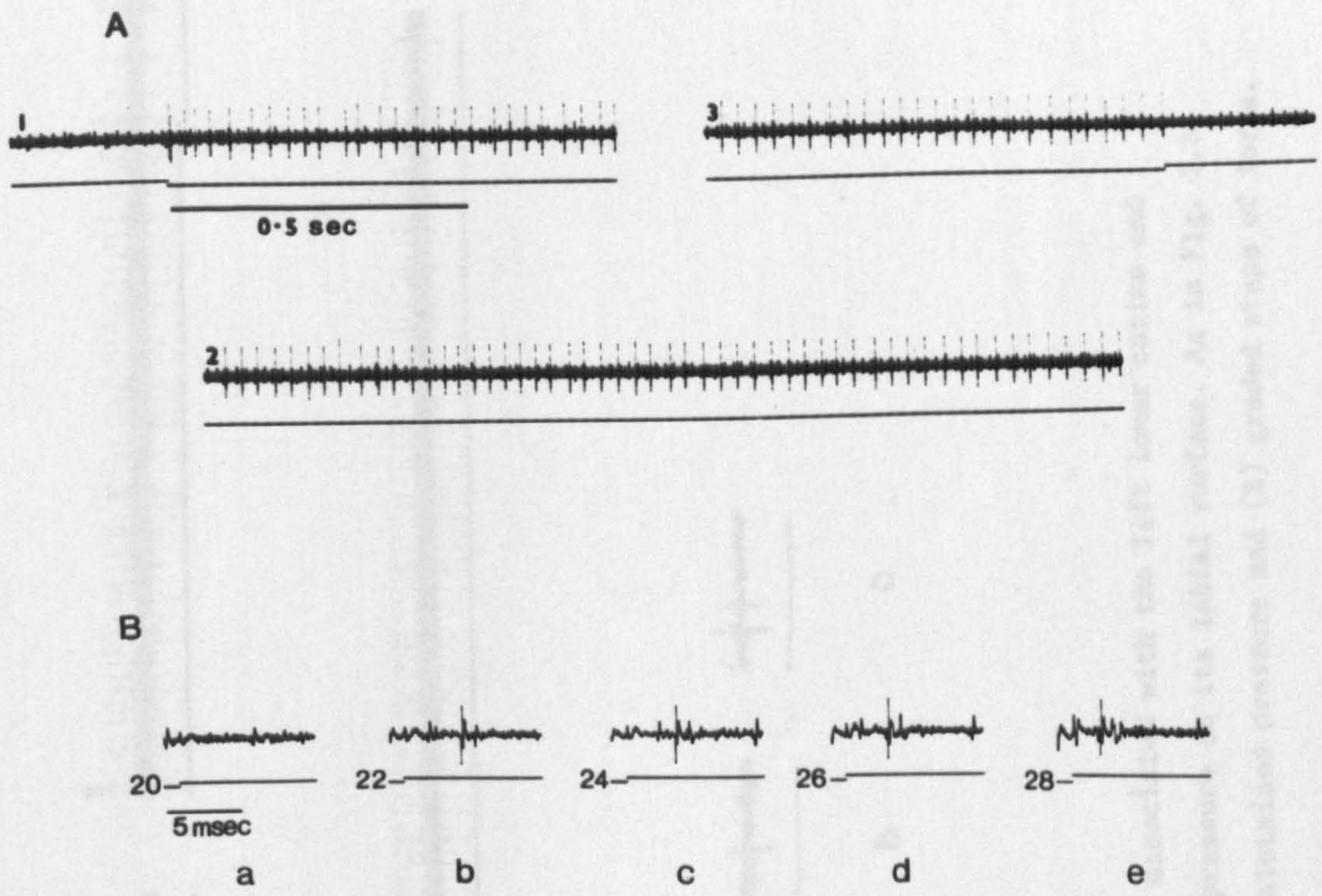

Fig. 2.8

The behaviour of a dental unit associated with the left upper canine and preferentially activated by pressure on its labial surface.

A. Responses to prolonged, slightly suprathreshold pressure. 1,2 and 3 are sequential parts of a continuous record. Downward deflexion of the lower trace indicates application of pressure.

B. Effect of increasing stimulus amplitude. Force is shown in arbitrary units. Upward deflexion indicates application of pressure.

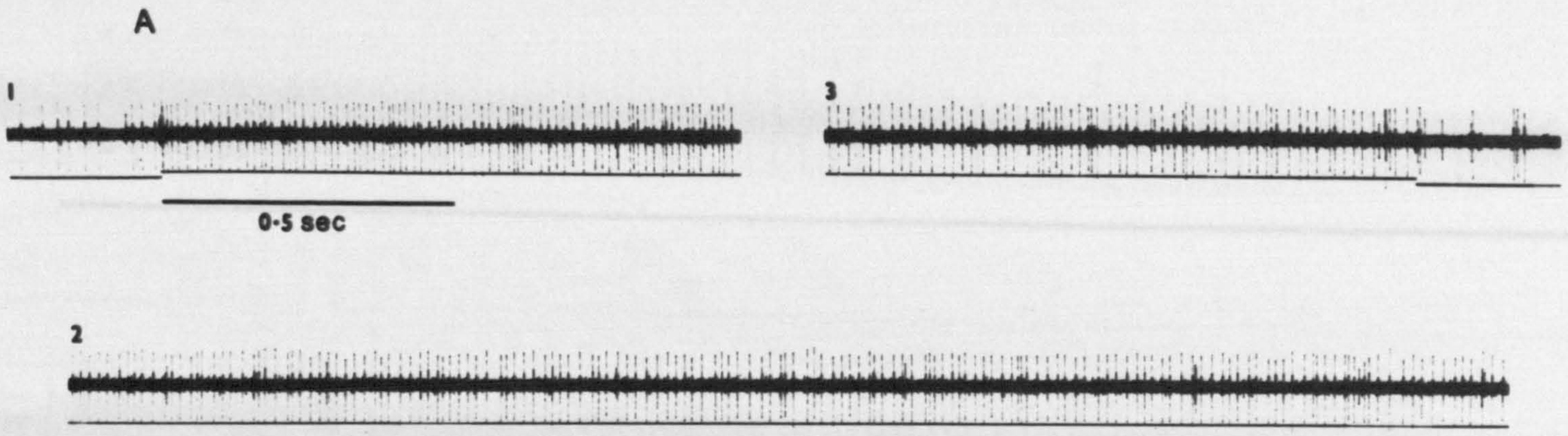

Fig. 2.9

Responses of a dental unit associated with the left lower canine and preferentially activated by pressure on its labial surface. As in Fig. 2.8 (A) shows discharge during maintained pressure and (B) graded steps of force.

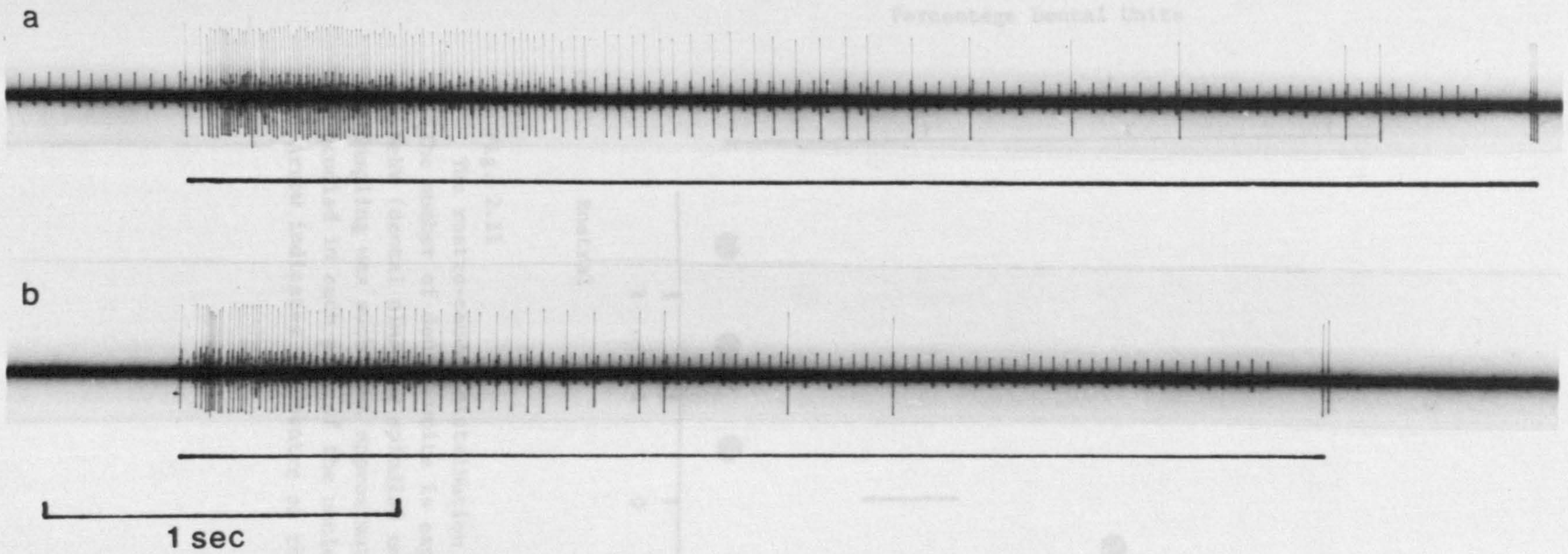

Fig. 2.10

Responses of a dental mechanoreceptor unit (large action potentials) and a jaw muscle spindle unit (small action potentials) recorded from the same site in the MeNV. In (a) the spindle afferent cell was activated by jaw opening prior to the application of pressure to the tooth associated with the dental unit. Duration of pressure is indicated by the bar. In record (b) jaw opening and tooth pressure were applied almost simultaneously.

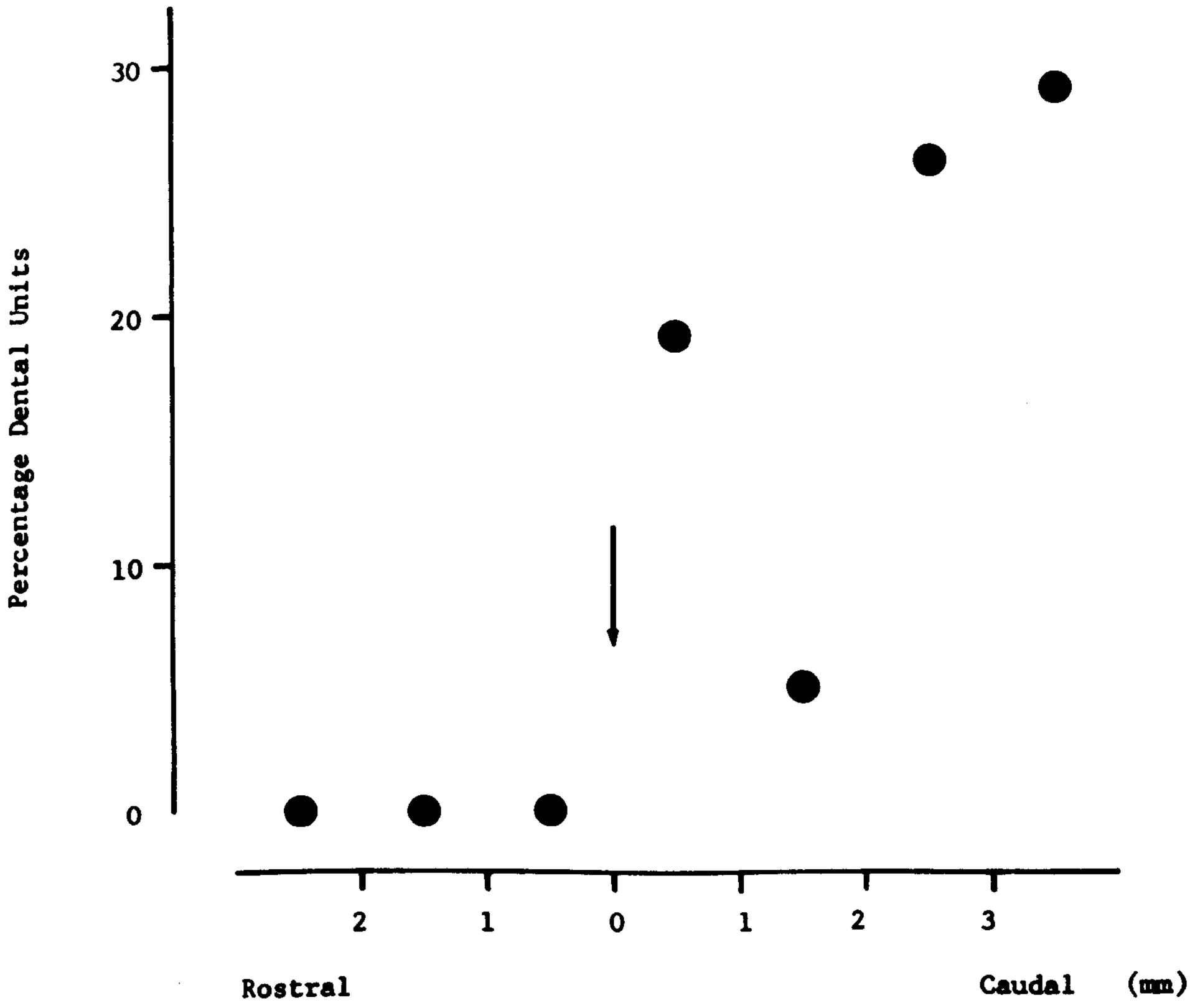

Fig. 2.11

The rostro-caudal distribution of dental units within the MeNV. The number of dental units is expressed as a percentage of the total MeNV (dental plus jaw spindle) units located in any given region. Sampling was such that approximately equal numbers of units were studied in each part of the nucleus.

Arrow indicates the centre of the superior colliculus.

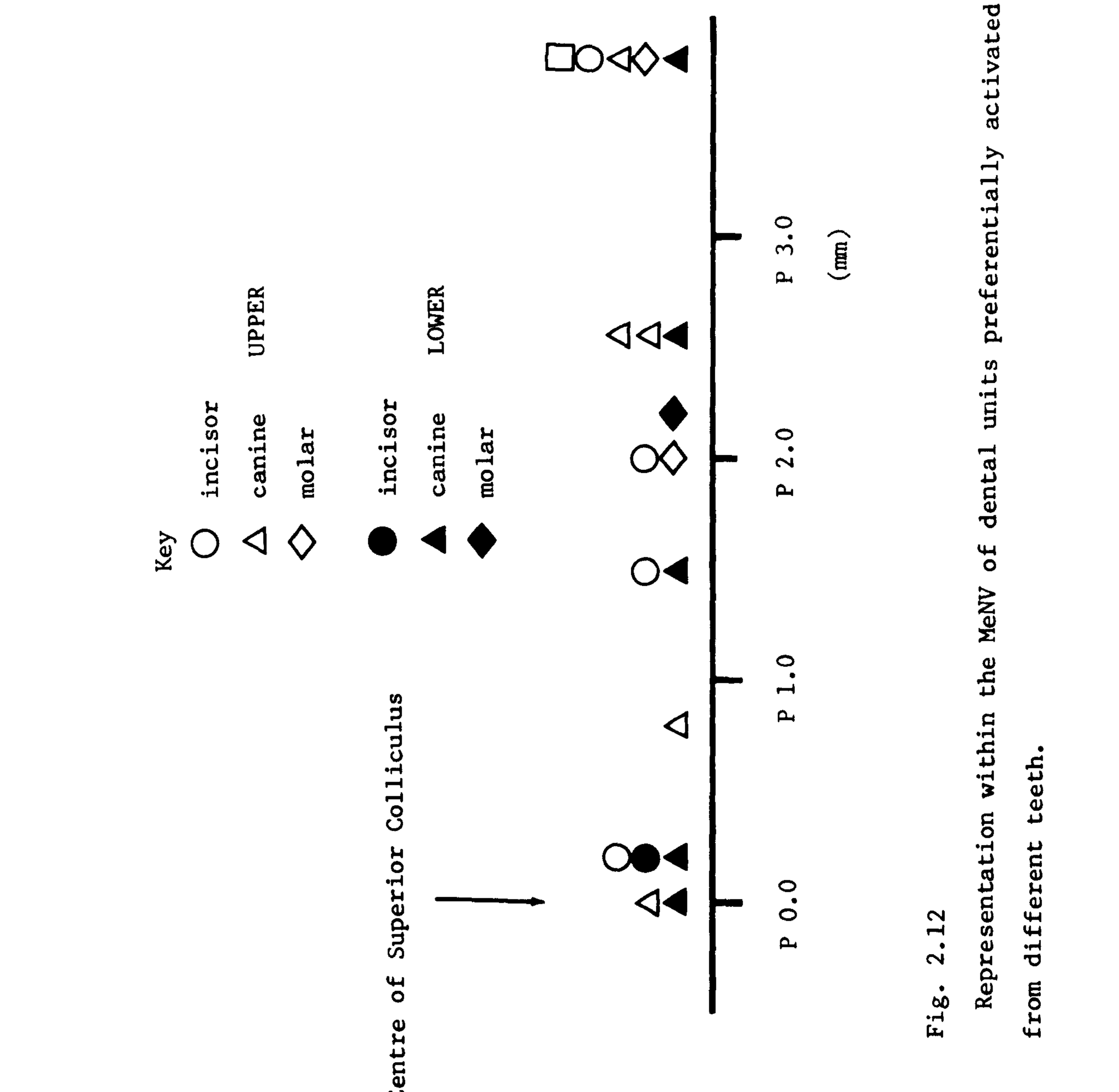

Fig. 2.12

Representation within the MeNV of dendritic units preferentially activated from different teeth.

2.4.2 Jaw Opening Units

All of the cells related to jaw movements were activated by opening, never by closing. This suggests that the receptors concerned were probably either stretch receptors in the jaw-closing muscles or T.M.J. receptors. The latter possibility is unlikely in view of their unidirectional sensitivity. Furthermore, these cells could always be excited by light pressure on one of the jaw muscles with the jaw held rigidly. Joint receptors were eliminated in all cases in this way and by more positive tests for muscle spindles described below.

2.4.3 Evidence for First Order Nature of Cells

Post mortem examination of brains (mainly from the earlier experiments) often permitted the identification of electrode tracks and indicated that recording was made from the region of the MeNV. Unitary activity was inevitably extremely resistant to anaesthesia and could persist for several minutes after respiration had been arrested by pentobarbitone. Also cells showed little sign of injury by the repeated close passage of the electrode tip suggesting the absence of a dendritic tree. In those cases where other, unrelated, activity was present, jaw-opening units invariably gave extracellular action potentials of greater amplitude, as is to be expected from large first order somata. Latency measurements were not attempted because of the difficulty in exposing the appropriate nerves without widespread damage of the jaw muscles and because conduction distances are uncertain.

2.4.4 Distinction of Cells Belonging to Muscle Spindles and Tendon Organs

Passive stretch of a muscle can excite both muscle spindles and Golgi tendon organs. These two types of receptor are conventionally distinguished by their responses during muscle contraction.

The immediate classification of the majority of jaw opening units as muscle spindle afferents was possible from their behaviour during twitches. Typical records are shown in Fig. 2.13. The large artifacts at the beginning of the records due to synchronous muscle action potentials could not be avoided, but do not obscure the spindle-like responses of the units, i.e. cessation of firing during the rising phase and a burst of impulses during the falling phase of contraction.

Occasionally, contraction of one of the jaw-closing muscles produced speeding, suggesting a tendon organ in that muscle. However, closer examination, using local pressure, always revealed that the muscle being stimulated was not that in which the receptor was situated. In fact the true muscle of origin of the afferent was being stretched by the contraction of one of its neighbours. This excitation can arise since the jaw-closing muscles have opposing effects in lateral sliding motions at the T.M.J. The extent of such lateral movements is small but sufficient to activate spindles. Contraction of the masseter primarily produces jaw closing, but also some lateral deflexion which in turn can passively stretch the pterygoid.

These conclusions were supported by the responses of a limited number of units tested with intracarotid arterial, and a larger

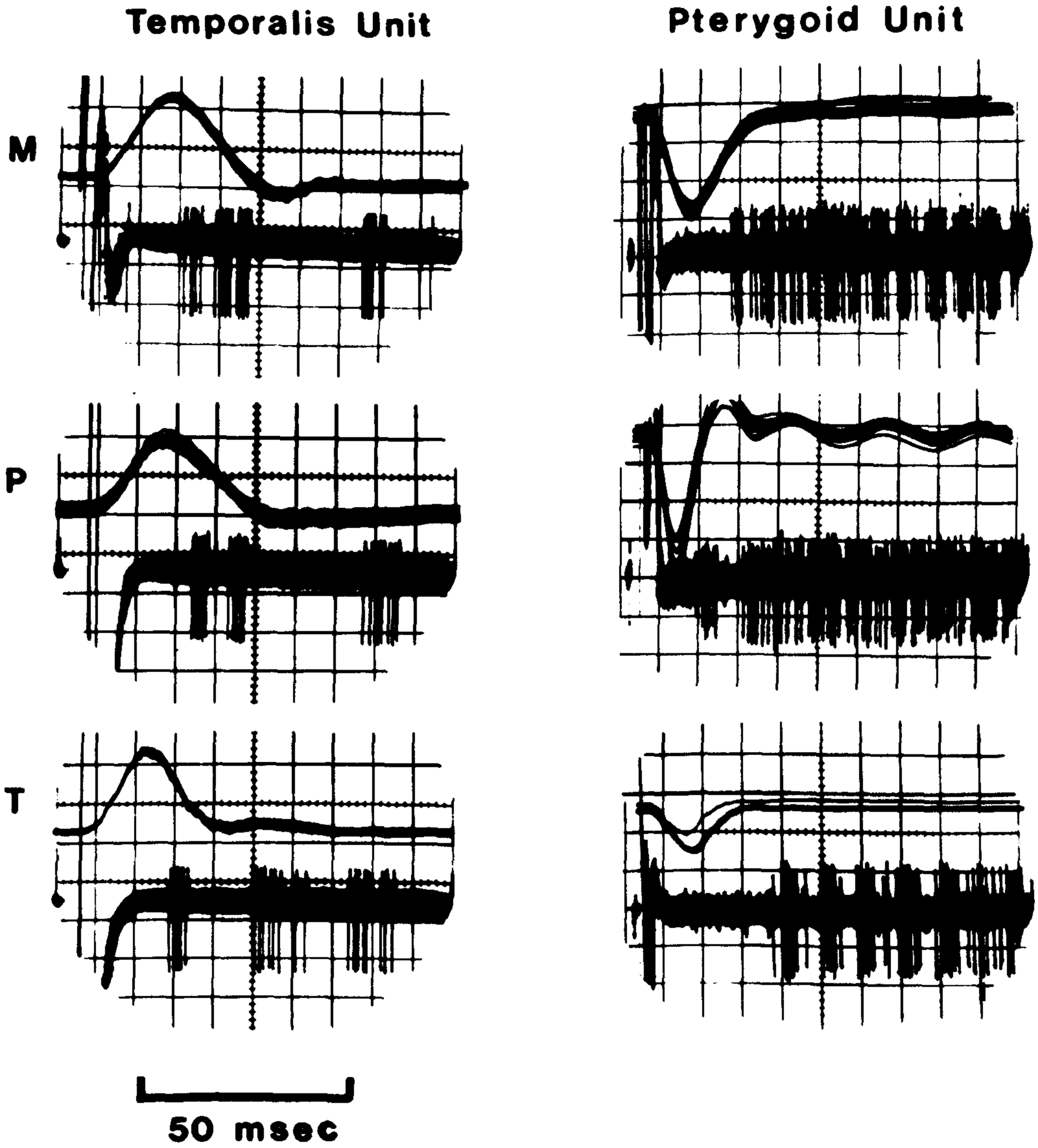

Fig. 2.13

The responses of two MeNV units, to twitches of the masseter (M), medial pterygoid (P) and temporalis (T) muscles. These units were believed to be associated with spindles in the temporalis and pterygoid muscles respectively. Upper trace indicates jaw displacement. For the temporalis unit jaw closing is represented by an upward defexion and for the pterygoid by a downward deflexion. Each record was obtained by superimposing ten individual responses. Contractions were against a weak spring.

(from Cody, Lee & Taylor, 1972)

number with intravenous injection of SCh. Activation was consistently produced, a characteristic of spindles not found with tendon organs (Granit, Skoglund & Thesleff, 1953). In addition, in preliminary experiments, under light anaesthesia, conspicuous changes in firing resulted from pinna twisting, presumably due to fusimotor excitation of spindles (Granit, Job & Kaada, 1952).

2.4.5 Distribution of Spindle Units According to Muscle of Origin

The muscle of origin of cell bodies of spindle afferents were identified by (a) application of surface pressure, (b) electrical stimulation, (c) manipulation of the mandible and (d) pressure on the eyeball.

It is well-known that the application of force to the surface of a muscle can produce sufficient deformation to stimulate spindles, especially primary endings. This method alone usually allowed confident localization, one muscle being far more sensitive to probing than the others.

In addition it was possible to take advantage of the differences in the mechanical arrangement of the jaw muscles to help determine the site of the receptors. Straightforward lowering of the mandible stretches the masseter, pterygoid and temporalis proportionately, whereas mediolateral deflexion has differential effects. Lateral movements stretch the pterygoid whilst medial movements stretch principally the masseter and to a lesser extent the temporalis.

Another helpful test was eyeball pressure. The bony orbit of the cat is incomplete posteriorly and therefore pressure on the eye is transmitted back to the pterygoid.

Muscle spindle afferent cells from each of the jaw closing muscles were found in all regions of the MeNV (Fig. 2.14). Applying the chi^2 test, no differences in relative rostro-caudal distribution of units from the three muscles could be detected. No attempt was made to determine the medio-lateral distribution because of the narrowness of the nucleus.

2.4.6 Extraocular Muscle Proprioceptors

In the course of recording from over 500 cells in the MeNV no single unit could confidently be identified as an extraocular muscle stretch receptor afferent.

Often cells were encountered which were sensitive to eye movements but invariably proved to be non-specific to the direction of rotation. Also such units were more affected by eyeball pressure, which tends to unload the extrinsic eye muscles, than by traction. All of these units proved to be extremely sensitive to jaw opening and to be excited by local pressure on a jaw muscle. Without exception such units were associated with jaw muscles, especially the medial pterygoid.

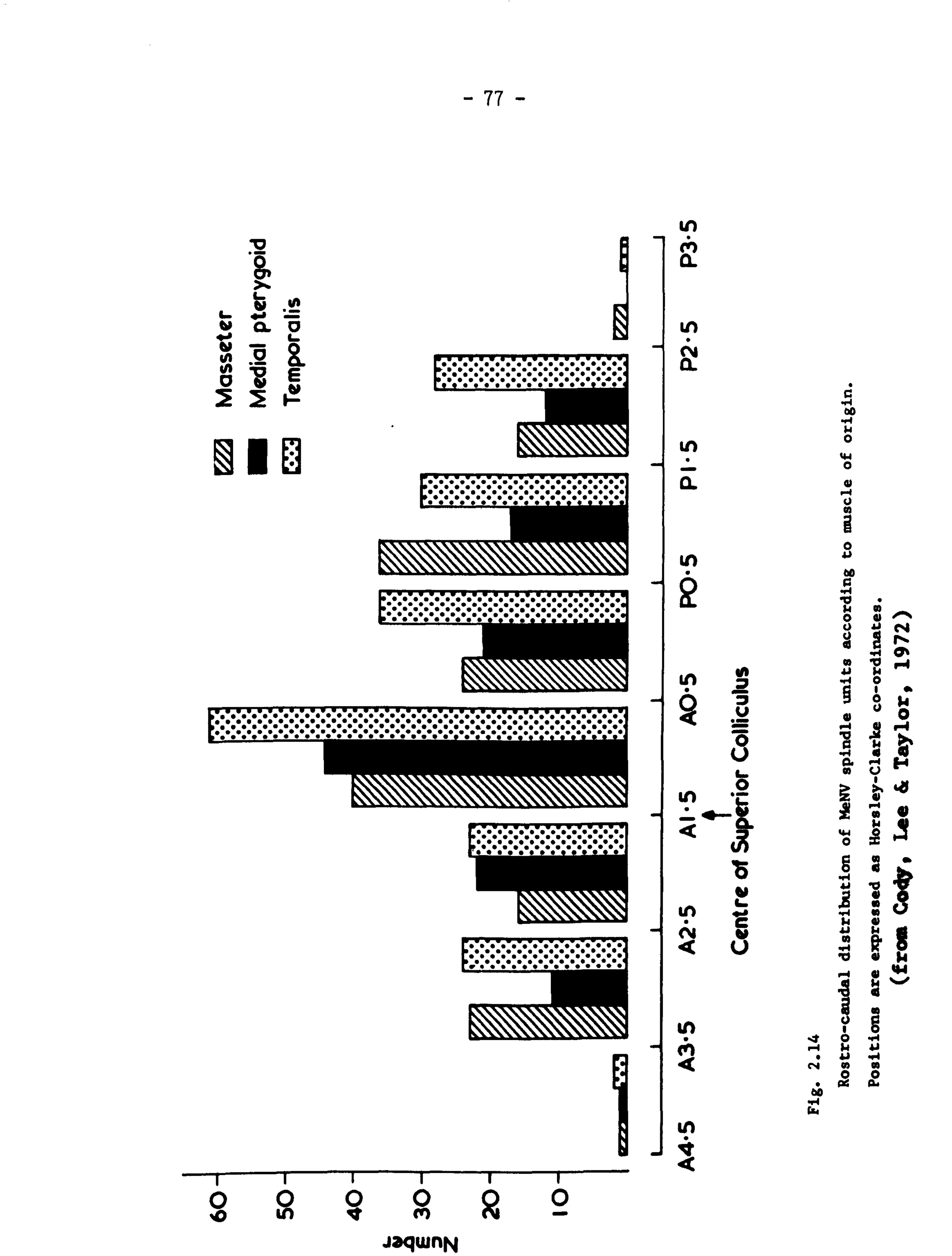

Fig. 2.14 Rostro-caudal distribution of MeNV spindle units according to muscle of origin.
Rostro-Caudal positions are expressed as Horsley-Clarke co-ordinates.
(from Cody, Lee & Taylor, 1972)

2.5 DISCUSSION

The presence of only two of the various cell types thought, at times, to be represented in the MeNV has been confirmed. These are the somata of ipsilateral dental mechanoreceptors and of muscle spindles of ipsilateral jaw closing muscles. The first order nature of these cells was supported by their resistance to anaesthesia, the apparent absence of a dendritic tree and the large size of their extracellular action potentials.

In the case of dental receptor units responses were of short latency and were generally similar in form to those recorded from the peripheral nerve (Pfaffman, 1939). The principal difference was that no spontaneously active dental units were encountered in the MeNV, although these were common in the inferior dental nerve (Pfaffman, 1939; Hannam, 1969).

The presence of dental mechanoreceptor afferent cells, activated from either single or several adjacent ipsilateral teeth, agrees with the findings of Jerge (1963), who classified such units into types I and II respectively. As in the present study, Jerge (1963) failed to find any spontaneously active dental units.

The principal difference between the work of Jerge (1963) and the present study concerns the distribution of dental units within the MeNV. In both studies dental units were found mainly in the caudal part of the MeNV, although according to Jerge their distribution is wider, extending to the rostral half of the nucleus. These discrepancies may have arisen from Jerge's reliance on exclusively stereotaxic means, in the intact brain, for localization

and from the insertion of electrodes at 30°. In contrast in the present work electrode penetrations were made vertically, after removal of part of the cortex and visualisation of the superior colliculus. The latter conditions, in my experience, are more reliable. Recently more extensive sampling of dental units has also shown them to be restricted to the caudal part of the cat MeNV (Cody, Harrison, Taylor & Weghofer, 1974).

Although not studied in detail, there was no evidence of activation of any MeNV cells by adequate contralateral stimulation, even in lightly anaesthetized animals. This contrasts with the bilateral representation of spindle afferents proposed by Smith, Marcarian & Niemer (1967). However, the published records of these workers show some surprising features. Supposed ipsilateral and contralateral evoked potentials, recorded simultaneously during stretching of jaw muscles separated at their insertion, were attributed, respectively, to first and second order neurones despite almost identical form and latency (Smith, Marcarian & Niemer, 1967). Latency values are not quoted and no time scale is provided.

Unfortunately, because degeneration techniques are not readily applicable to the study of polyneuronal pathways, no reliable histological information is available concerning possible bilateral connexions.

Neither was any evidence found to support the representation of jaw closing muscle tendon organ afferent somata. Smith (1969) claimed to have shown increased discharge of stretch sensitive units during the rising phase of tension in muscle twitches. In his experiments stimulation was limited to the masseter muscle and

he did not appreciate that contraction of this muscle can excite spindles in the pterygoid. In the present work, when this form of indirect activation was taken into account, the behaviour of all units responsive to jaw opening was identified as that of muscle spindles. In addition the firing of such units was consistently speeded by SCh, a characteristic of spindles not found with tendon organs (Granit, Skoglund & Thesleff, 1953), and in lightly anaesthetized animals could be activated by fusimotor reflexes.

Anatomical studies (Szentagothai, 1948) also argue against tendon organ representation in the MeNV. Midbrain lesions cause degeneration of spindle but not of tendon organ afferents.

The most probable alternative site for tendon organ units is in the trigeminal ganglion. However, Beaudreau & Jerge (1968) failed to find them there, although it is possible that the use of passive muscle stretch was inappropriate in view of the relative insensitivity of these receptors to this form of stimulus (Jansen & Rudjord, 1964). It would be desirable to look for these proprioceptors while trying to excite them with muscle twitches.

Present observations also cast considerable doubt on the presence of eye muscle stretch receptors in the MeNV of the cat (Fillenz, 1955). It seems probable that previously confusion may have arisen because of the disturbance of masticatory muscle spindles by movements in the orbit, which in the cat has no postero-lateral wall. Recently recordings of cells activated by extraocular muscle stretch were made from the trigeminal ganglion in the pig and sheep (Manni, Bortolami & Dosole, 1966; 1968). In these animals section of the ophthalmic nerve abolished the trigeminal ganglion response.

This finding agrees well with anatomical evidence (see Hosokawa, 1961) suggesting that eye muscle proprioceptive afferents run in the ophthalmic division of the trigeminal nerve. This is particularly well seen in the goat in which Whitteridge (1955) and Cooper & Daniel (1957) found separate purely sensory bundles passing from the eye muscle nerves to the fifth nerve.

A good case for the existence of eye muscle proprioceptor neurones within the brainstem is found in the goat (Cooper, Daniel & Whitteridge, 1953). However, responses were recorded over a wide area and their latencies of 20-50 msec can hardly be considered convincing evidence of the cells concerned being first order. Midbrain activity in the sheep, recorded from comparable sites, has recently been attributed to second order cells (Manni, Palmieri & Marini, 1972).

2.6 SUMMARY

1. Two types of units were identified in the MeNV, namely ipsilateral jaw closing muscle spindle afferent cells and ipsilateral dental mechanoreceptor afferent cells.

2. Muscle spindle units were found throughout the rostrocaudal extent of the MeNV. Such units were not segregated according to muscle of origin (i.e. masseter, pterygoid or temporalis).

3. Dental units were restricted to the caudal part of the nucleus.

4. No evidence was found of either tendon organ or eye muscle proprioceptor representation.

5. The projection to the nucleus appeared to be exclusively ipsilateral.

SECTION 3 CLASSIFICATION OF JAW-CLOSING MUSCLE SPINDLES

3.1 INTRODUCTION

Although the work of Corbin & Harrison (1940) and Jerge (1963) demonstrated the presence of jaw muscle spindle afferent cells in the MeNV no attempt was made to quantify their responses to stretch or to classify them on this basis.

Evidence from other muscles, largely those of the hindlimb, suggests characteristic differences between the properties of the two types of spindle sensory ending, namely primary and secondary endings (see Section 3.2; Matthews, 1972, Chpt.4). However, some doubt remains as to whether these represent truly functionally distinct populations or belong to a single continuum.

The cat jaw-closing muscles, which have been shown histologically to contain "typical" primary and secondary endings (Szentagothai, 1948), were studied with these questions in mind. This provided an opportunity of testing the generality of findings in the cat limb muscles. Also the method of recording from cell bodies was thought to be more likely to provide representative sampling than that of splitting dorsal root filaments, which can introduce bias according to fibre size.

3.2 HISTORICAL REVIEW

Matthews, B.H.C. (1933) first demonstrated the characteristic silencing of spindle afferents during twitch contractions and their speeding during passive stretch. In those afferents studied the response to stretch was related to the rate of muscle lengthening.

He also attempted functionally to subdivide spindle afferents into groups corresponding to the two histologically recognized forms of sensory ending (Ruffini, 1898), mainly on the basis of their response to fusimotor stimulation. Some endings (A2) showed an increased discharge frequency whilst others (A1) were comparatively unaffected.

Hunt (1954) showed that spindle afferents of the cat soleus, isolated from dorsal roots, fell into two groups according to conduction velocity. The more rapidly conducting Ia fibres had a slightly lower threshold to stretch and were believed to be associated with primary endings, whereas the group II fibres were presumed to terminate as secondary endings.

Subsequently Cooper (1959; 1961) demonstrated, in decerebrate cats, that the "dynamic" sensitivity to rate of stretch earlier described by B.H.C. Matthews, was a property only of Ia afferents. This difference between Ia and II fibres was substantiated by Harvey & Matthews (1961) using a wide range of constant velocity stretches in the de-efferented cat soleus, and extended to include various other muscles, e.g. tenuissimus (Bessou & Laporte, 1962), gastrocnemius (Renkin & Vallbo, 1964) and tibialis anterior (Alnaes, Jansen & Rudjord, 1965).

Further differences in the passive properties of primary and secondary afferents were later found. Ia fibres, in contrast to II fibres, can be activated by very small amplitude vibrations and may be driven at high frequencies in this way (Bianconi & van der Meulen, 1963; Brown, Engberg & Matthews, 1967). This agrees well with the previous observation that primary afferents are far more sensitive to brief stretches than secondaries (Lundberg & Winsbury, 1960). Also in

de-efferented and decerebrate preparations group II fibres provide a far more regular discharge at constant muscle length (Pascoe, 1965; Stein & Matthews, 1965).

3.3 METHODS

Attempts were made to distinguish primary and secondary spindle afferents by the following tests:-

a) Dynamic Index (DI) of Crowe & Matthews (1964).

DI was measured as the difference in instantaneous frequency between the maximal value, corresponding to the attainment of final length, and that 0.5 sec. later, for four constant velocity stretches. Stretch was applied to the jaw muscles by ramp jaw openings of 1.5° amplitude, starting from 8.5°. Such movements extend the muscles by approximately 0.4-0.5% of their resting lenth or 3.5% of their physiological range. The velocities used were 1.0, 2.2, 3.25 and 4.5°/sec.

These stretches differ in two main respects from those commonly used in characterizing spindle afferents (see Matthews, 1963). Firstly, the final length of the muscle was 6-7*mm short of the maximum which can be attained in the body. Secondly, the velocities were relatively low. However, the greatest difference in DI/velocity curves for soleus primary and secondary endings is seen at low velocities (Matthews, 1963, Fig.11). A quotient was also derived of DI/velocity by linear regression and is referred to as "normalized DI".

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* For temporalis muscle.

b) Maximal frequency following (Brown, Engberg & Matthews, 1967). Small amplitude sinusoidal stretches were applied to the jaw at increasing frequencies up to 300 Hz. At low frequencies several impulses were discharged per cycle (Fig. 3.1). Above 20-30 Hz only one spike normally accompanied each stretching movement. The maximal frequency of stretching at which a unit would reliably fire one per cycle is referred to as the following frequency (FF).

c) Interspike interval variability (Stein & Matthews, 1965). The coefficient of variation (CV) of intervals, during maintained stretch at 10^{o} jaw opening, was calculated.

d) Amplitude of extracellular action potentials. Henneman, Somjen & Carpenter (1965) have suggested that the size of motorneurone axons reflect that of the somata. It seemed possible, therefore, that first order spindle cell bodies would fall into two groups according to size, corresponding to Ia and II fibres. Consequently the amplitude of extracellular action potentials of spindle units was measured. In a sufficiently large sample, individual bias according to electrode position might be minimized and a relationship between the size of somata and recorded action potentials emerge.

3.4 RESULTS

3.4.1 Effects of Anaesthesia

In a number of preliminary experiments spindle responses were recorded in lightly anaesthetized animals. Under these conditions fusimotor reflexes were present and stimuli such as pinna twisting (Granit, Job & Kaada, 1952) produced marked changes in unitary discharge. The

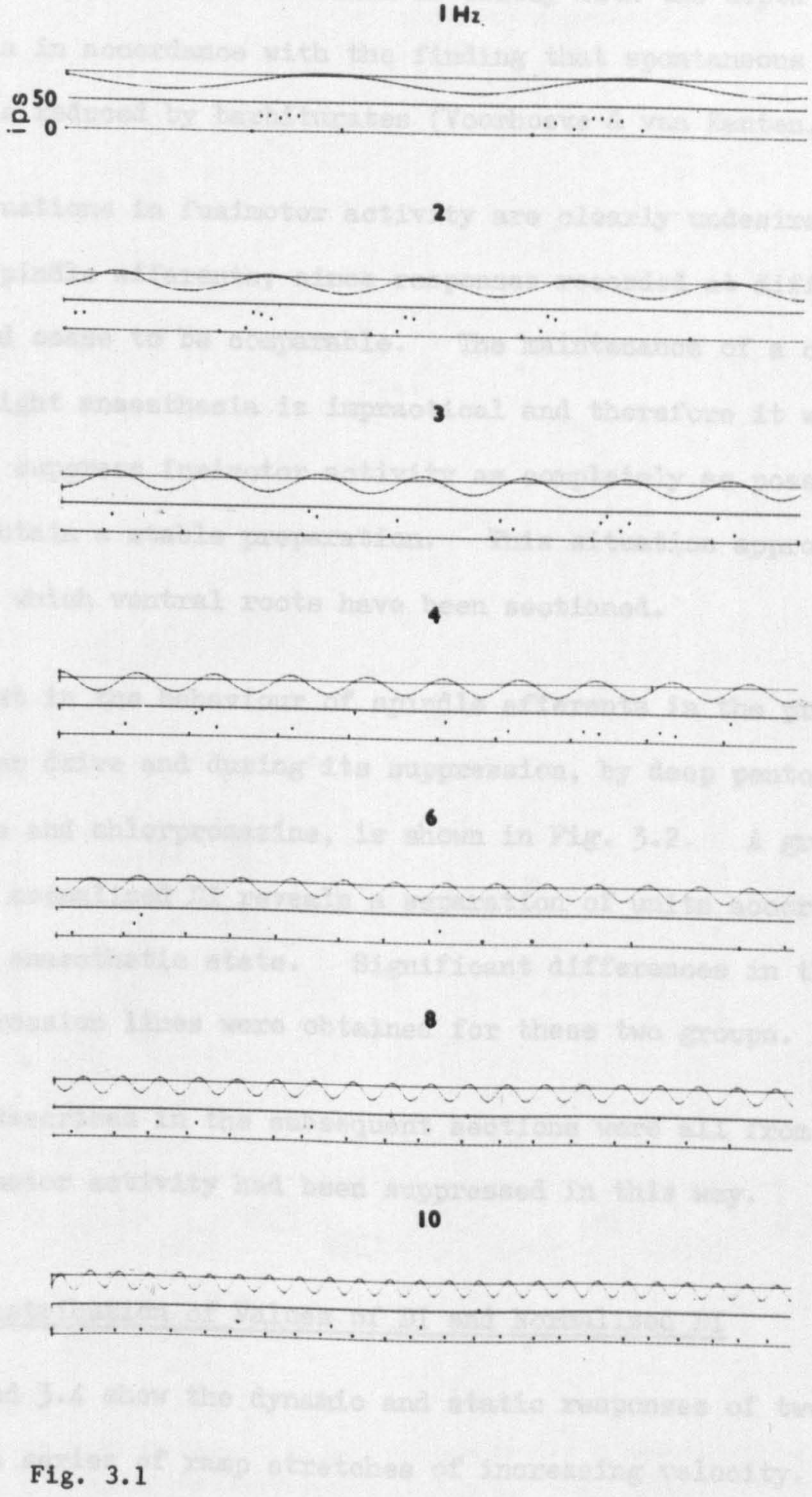


Fig. 3.1

Responses of a jaw muscle spindle unit, believed to originate in the masseter, to sinusoidal stretching at low frequencies. Each spike is represented by a dot, whose vertical deflexion is proportional to instantaneous frequency. The upper trace indicates jaw movement, opening being upwards. Amplitude of stretches were 3^{o}.

magnitude of these effects varied inversely with the depth of anaesthesia in accordance with the finding that spontaneous fusimotor activity is reduced by barbiturates (Voorhoeve & van Kanten, 1962).

Such fluctuations in fusimotor activity are clearly undesirable when sampling spindle afferents, since responses recorded at different times would cease to be comparable. The maintenance of a constant level of light anaesthesia is impractical and therefore it was decided to suppress fusimotor activity as completely as possible in order to obtain a stable preparation. This situation approximates to that in which ventral roots have been sectioned.

The contrast in the behaviour of spindle afferents in the presence of fusimotor drive and during its suppression, by deep pentobarbitone anaesthesia and chlorpromazine, is shown in Fig. 3.2. A graph of DI against normalized DI reveals a separation of units according to the prevailing anaesthetic state. Significant differences in the gradients of the regression lines were obtained for these two groups.

Responses described in the subsequent sections were all from animals whose fusimotor activity had been suppressed in this way.

3.4.2 Distribution of Values of DI and Normalized DI

Figs 3.3 and 3.4 show the dynamic and static responses of two spindle units for a series of ramp stretches of increasing velocity. The unit in Fig. 3.3 was extremely phasic with a large dynamic response, whereas the one in Fig. 3.4 was more static in behaviour. Occasionally a unit showed "saturation", i.e. DI was not linearly related to velocity but reached a maximum at one of the lower velocities of stretching. These units were excluded from further consideration.

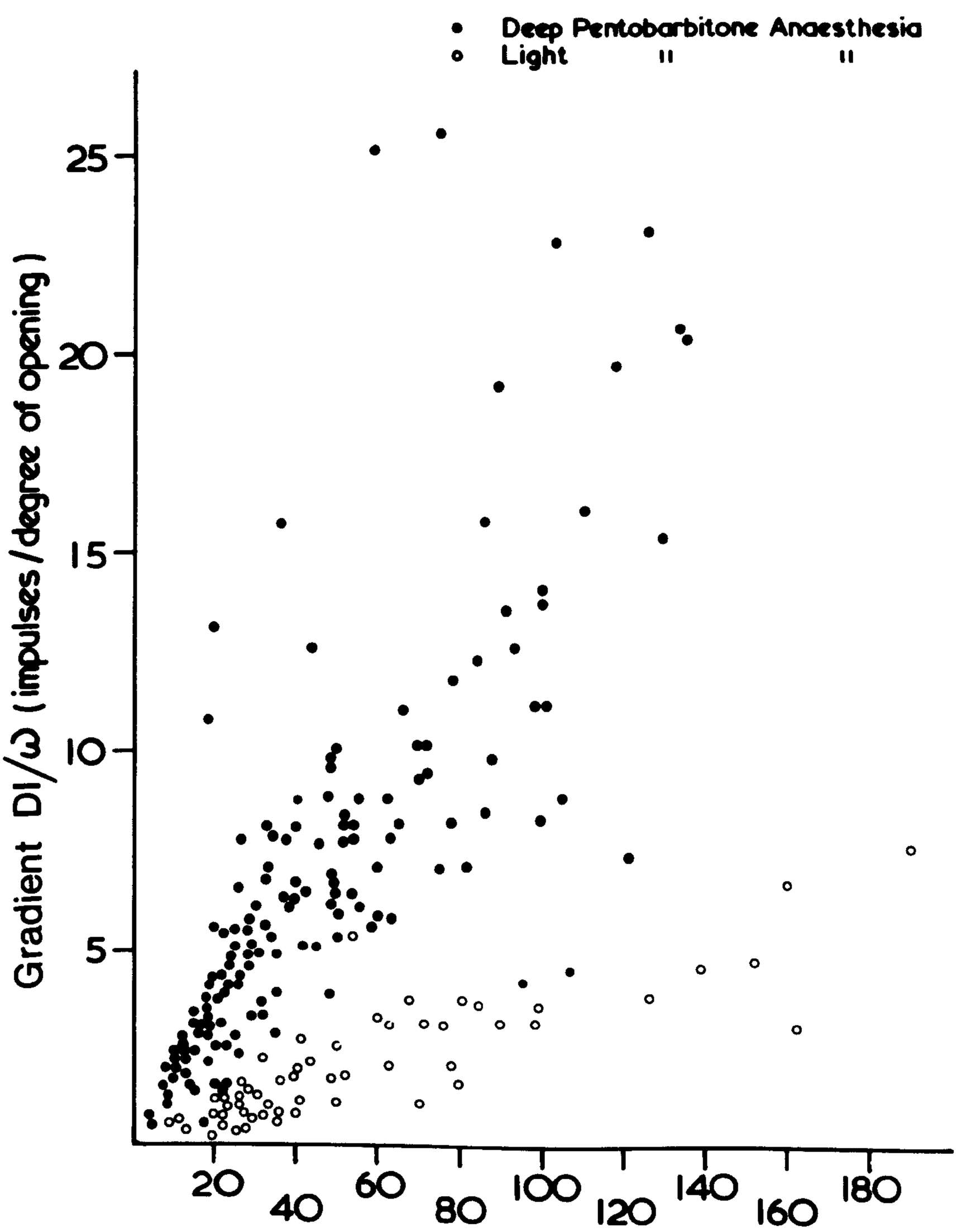


Fig. 3.2

Differences in the responses of jaw muscle spindle units to constant velocity stretching under conditions of light and deep pentobarbitone anaestesia. In the former situation fusimotor activation could be elicited by pinna twisting. In the later situation chlorpromazine was used, in addition to barbiturates, to suppress fusimotor activity. Normalised DI (ordinate) is plotted against DI at velocity 4.5^{o}/sec. (abscissa). Significant differences ($p = 0.005$) in the regression lines were obtained for the two groups of units pooled from the three jaw-closing muscles.

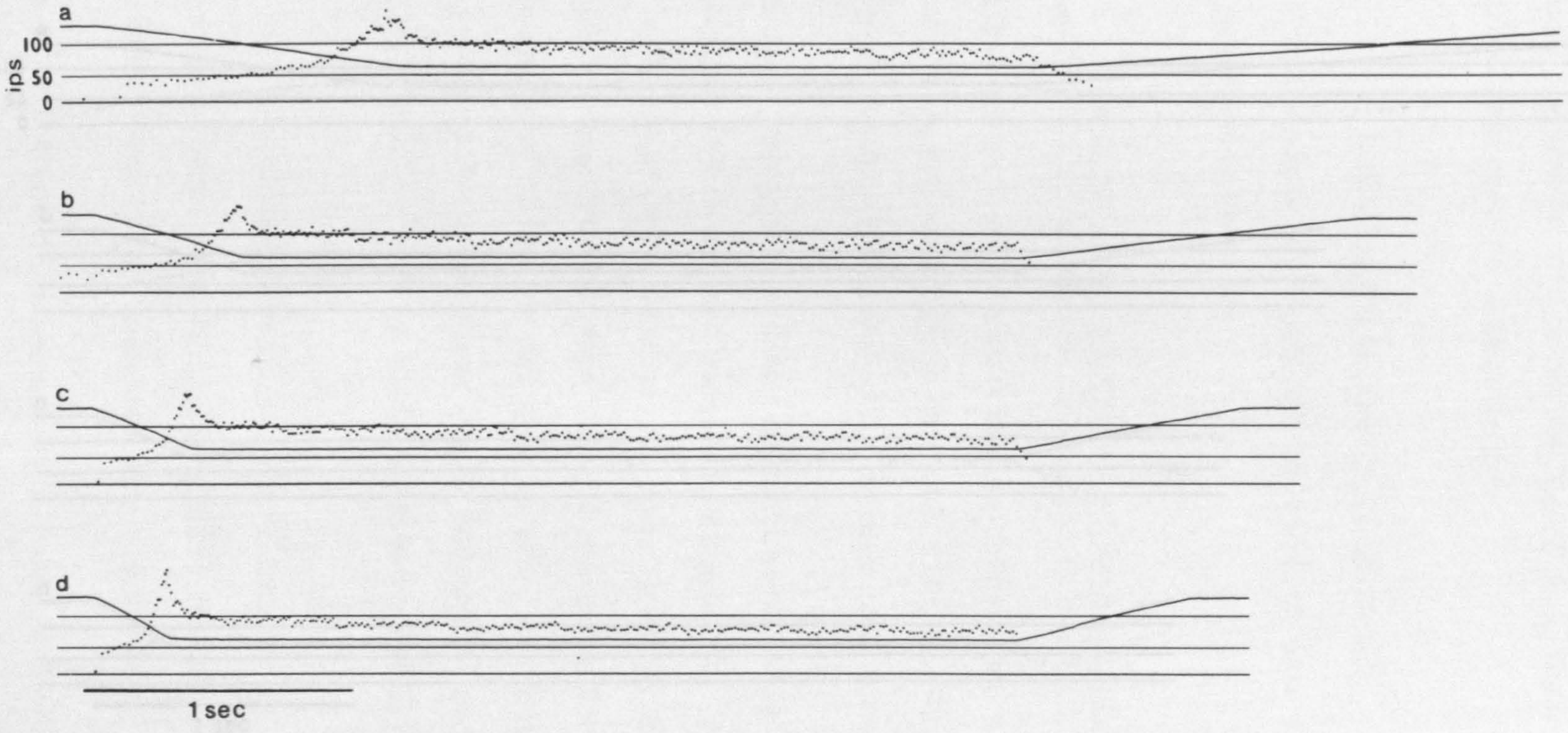


Fig. 3.3

Responses of a temporalis unit to ramp jaw openings (upper trace) of (a) 1.0, (b) 2.2, (c) 3.25 and (d) 4.5°/sec. Discharge is shown as instantaneous frequency. The relatively large dynamic response of the unit suggests that it is probably a primary.

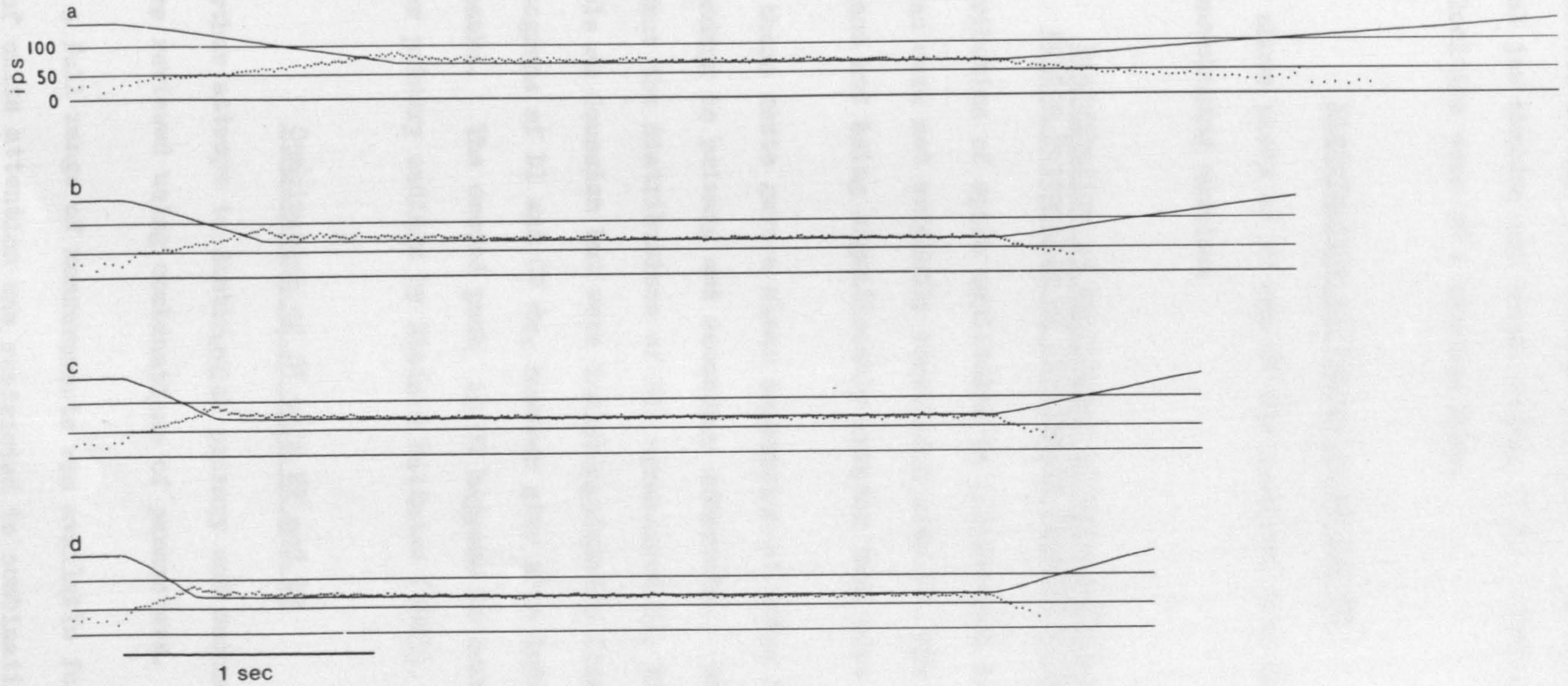


Fig. 3.4

Responses of a masseter unit to ramp jaw openings (upper trace) of (a) 1.0, (b) 2.2, (c) 3.25 and (d) 4.5°/sec. Discharge is shown as instantaneous frequency. The small dynamic response of the unit suggests that it is probably a secondary.

The distribution of DI measured at 4.5° jaw opening/sec and normalized DI are plotted in Fig. 3.5 for pooled data. Corresponding plots for individual jaw muscles are shown in Fig. 3.6. Histograms of DI at other velocities were of a similar form.

3.4.3 Distribution of Values of FF and CV

Fig. 3.7 shows plots of FF and CV for combined data from units in each of the jaw-closing muscles.

3.4.4 Distribution of Amplitudes of Extracellular Action Potentials of Jaw Muscle Spindle Units

The distribution of spike amplitudes is illustrated in Fig. 3.8. Spike amplitudes were not evidently correlated with DI, the correlation coefficient not being significantly greater than zero (Fig. 3.9).

None of these tests gave a clear separation of units into two groups corresponding to primary and secondary afferents. Statistical testing showed that the distributions of DI, normalized DI, FF and CV were not acceptable as Gaussian but were indistinguishable from lognormal. The histograms of DI and CV do, however give some indication of small second peaks. The second peak in CV happens to correspond to that found for primary endings by Stein & Matthews (1965).

3.4.5 Combination of DI with FF and CV

In a further attempt to distinguish primary and secondary afferents data were reviewed using combinations of parameters.

Because a full range of measurements was available for only a limited number of units attention was restricted to combinations of DI and CV,

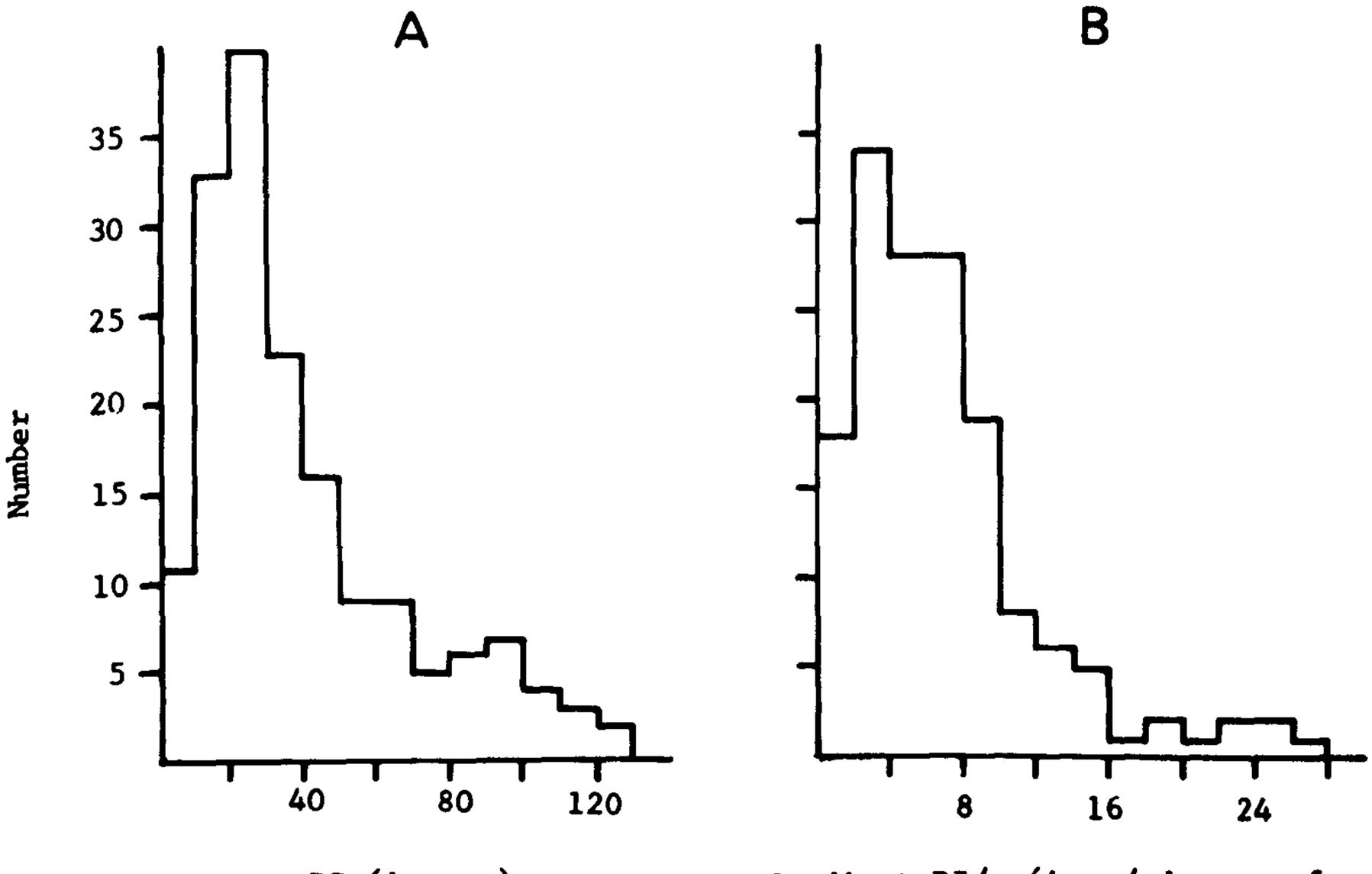
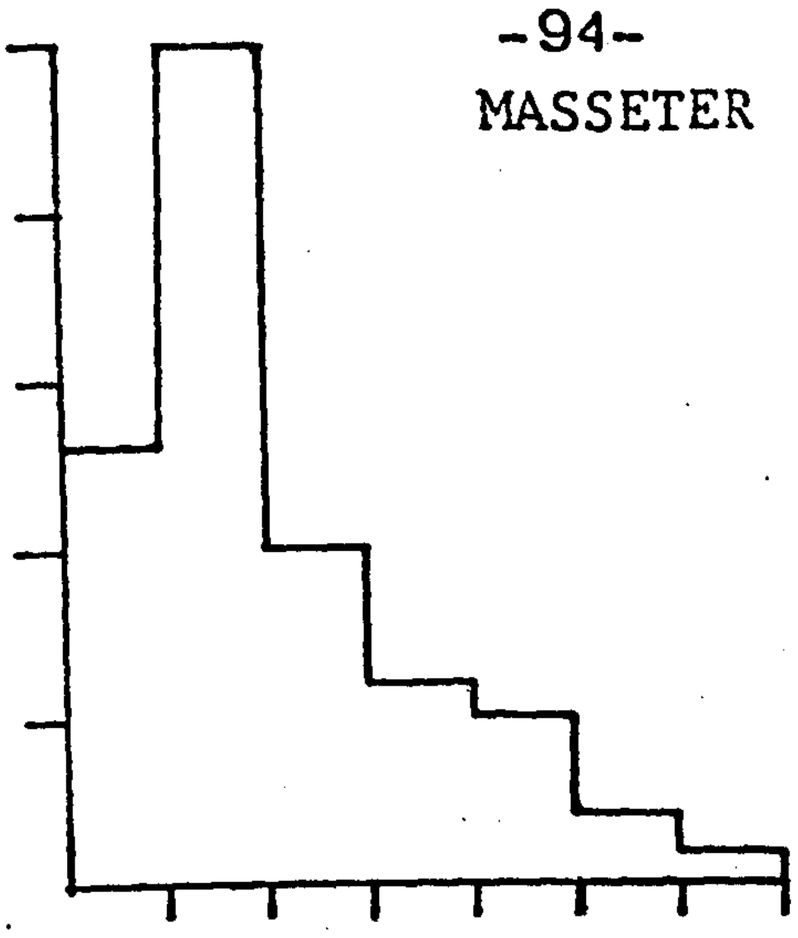
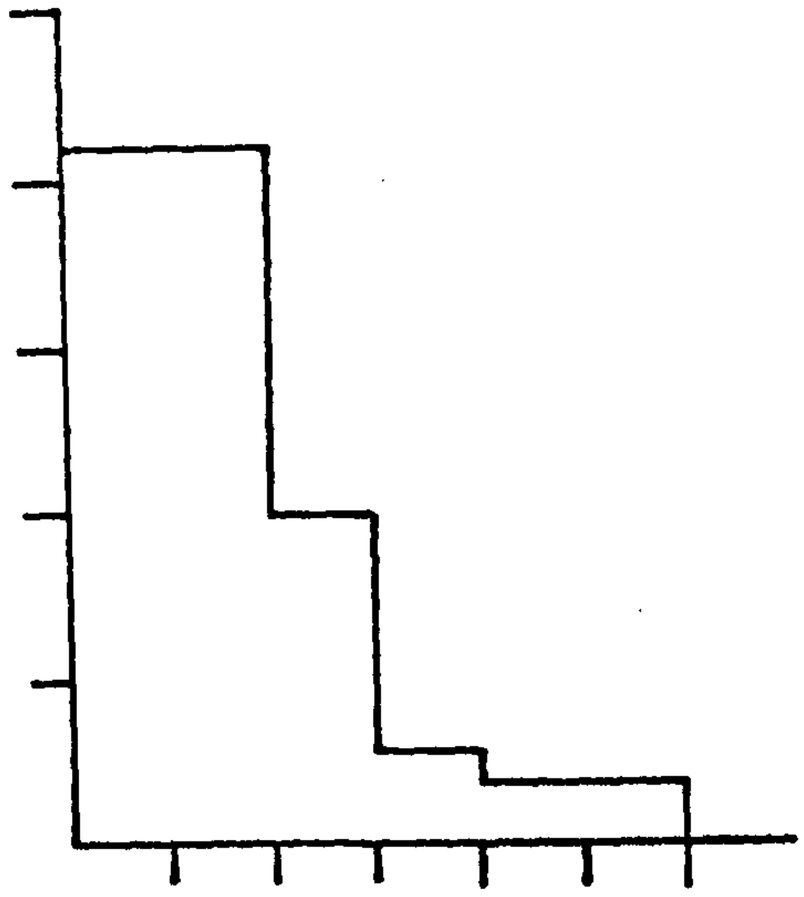


Fig. 3.5

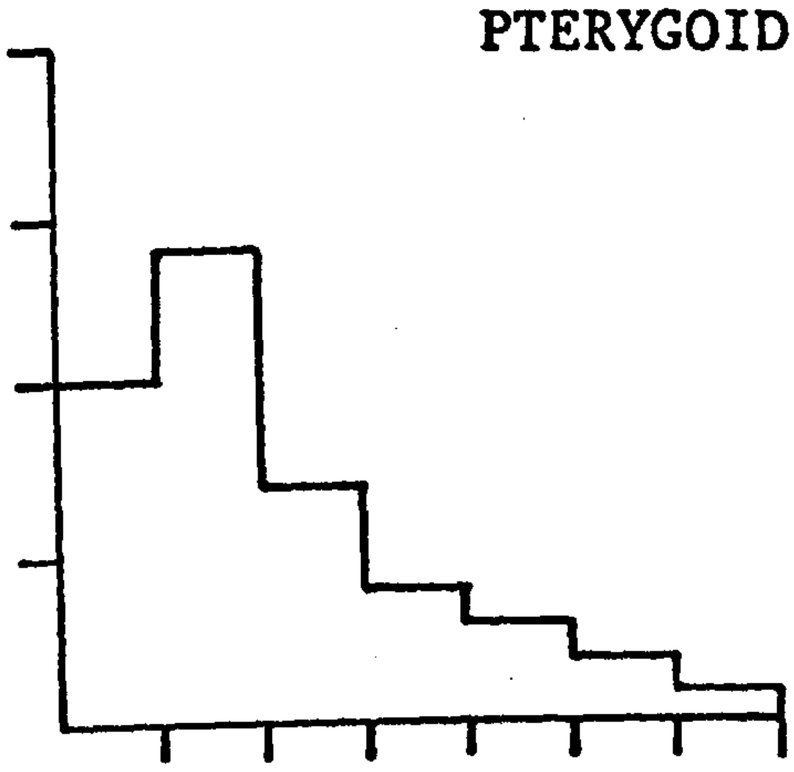
Distributions of values of (A) DI and (B) normalized DI for data pooled from the three jaw-closing muscles. DI was measured at stretching velocity 4.5^{o} jaw opening/sec.

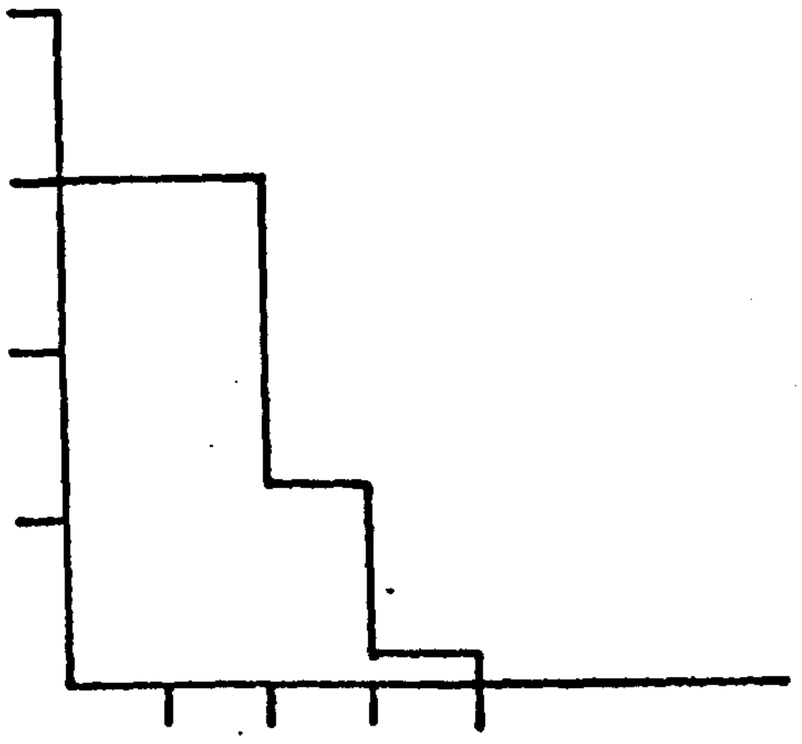
MASSETER



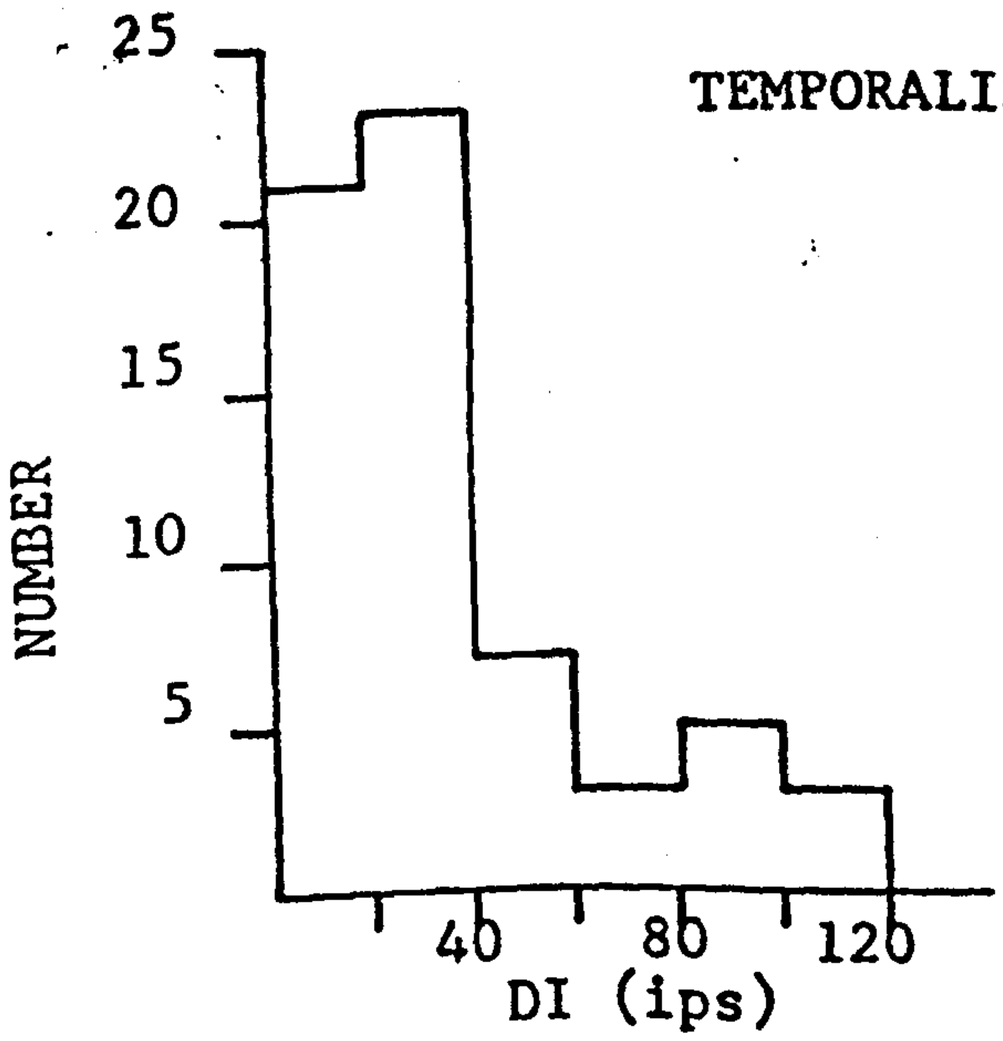


PTERYGOID





TEMPORALIS



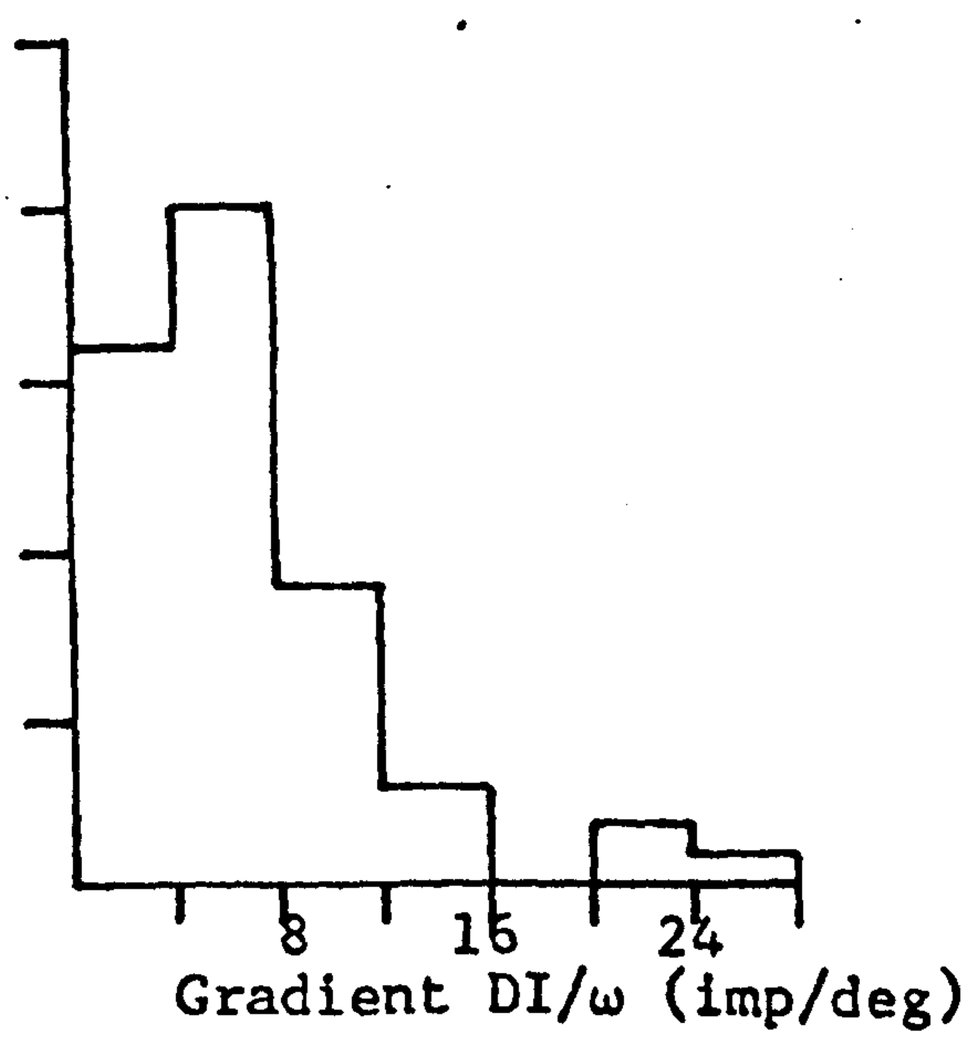


Fig. 3.6

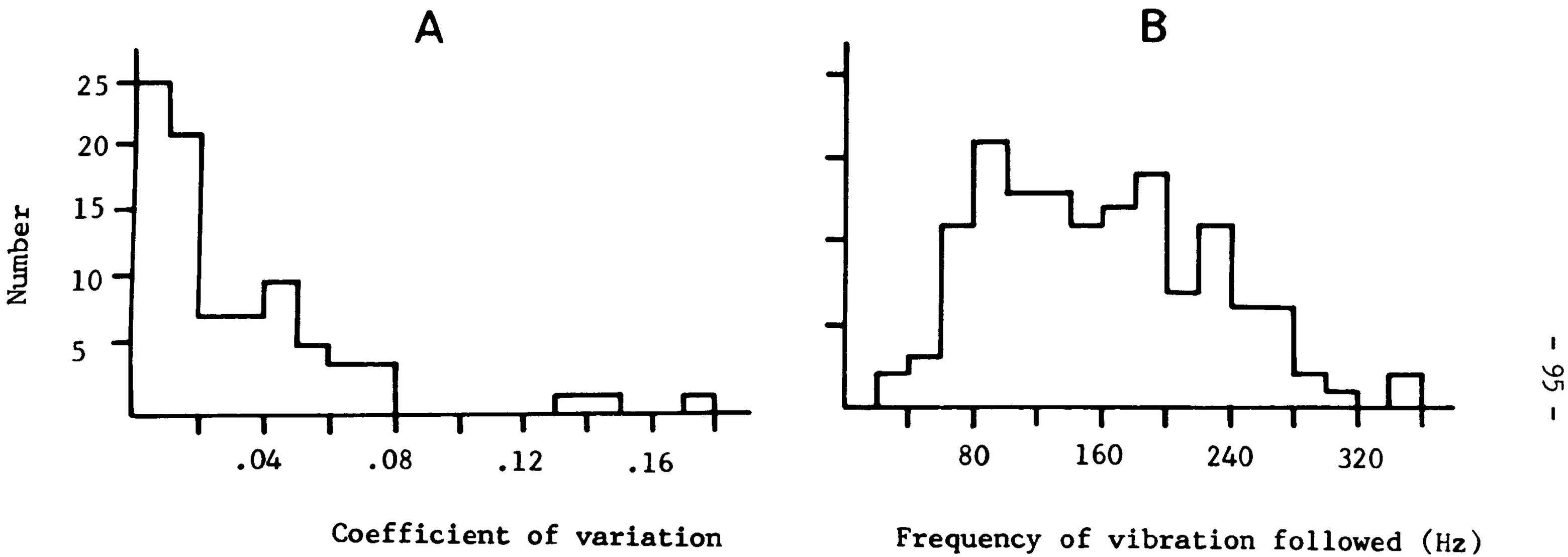


Fig. 3.7

Distribution of values of (A) CV and (B) FF for data pooled from the three jaw-closing muscles.

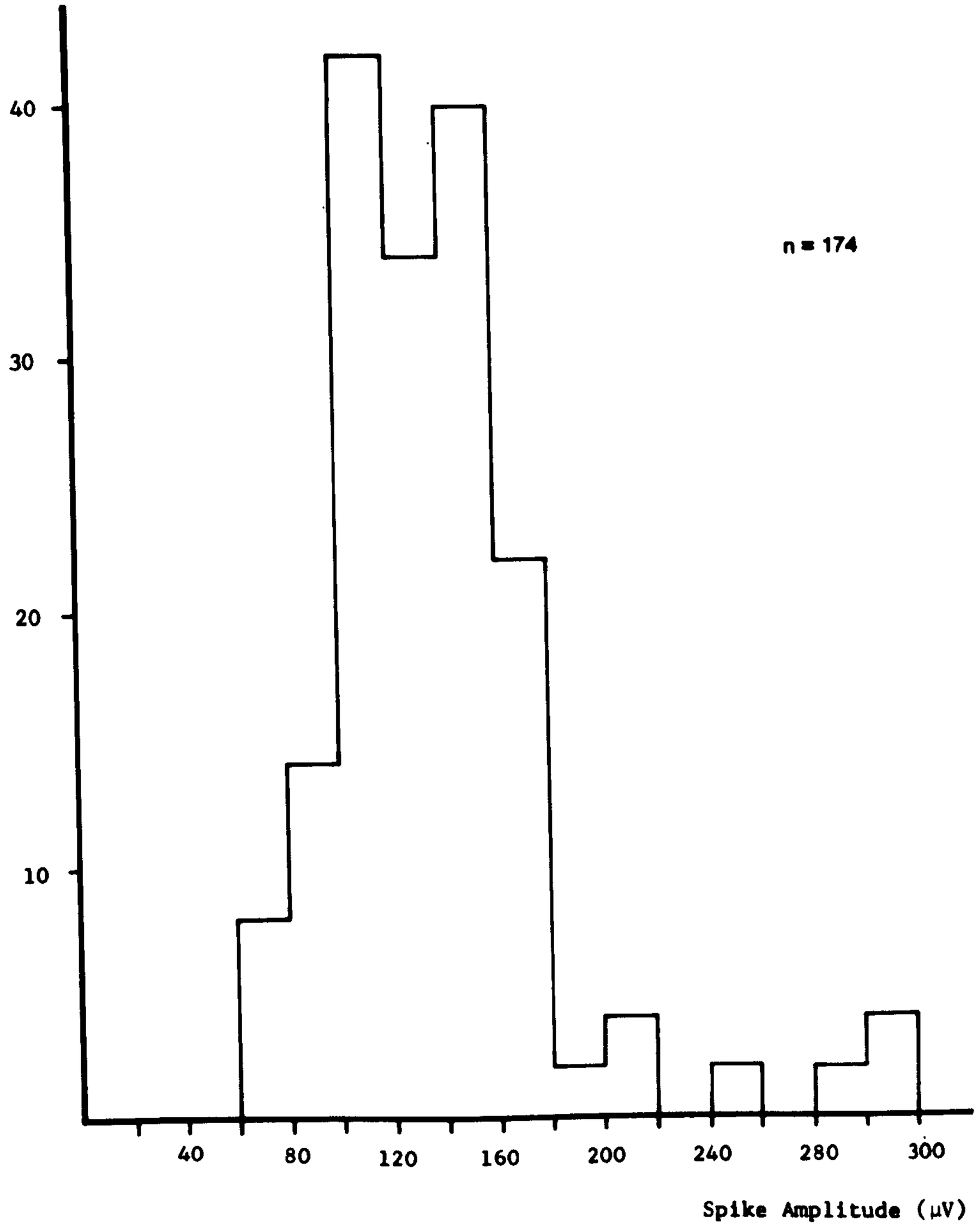


Fig. 3.8

Distribution of spike amplitudes of jaw muscle spindle units, pooled from the three jaw-closing muscles. Measurement was made from peak to peak of these biphasic extracellular action potentials.

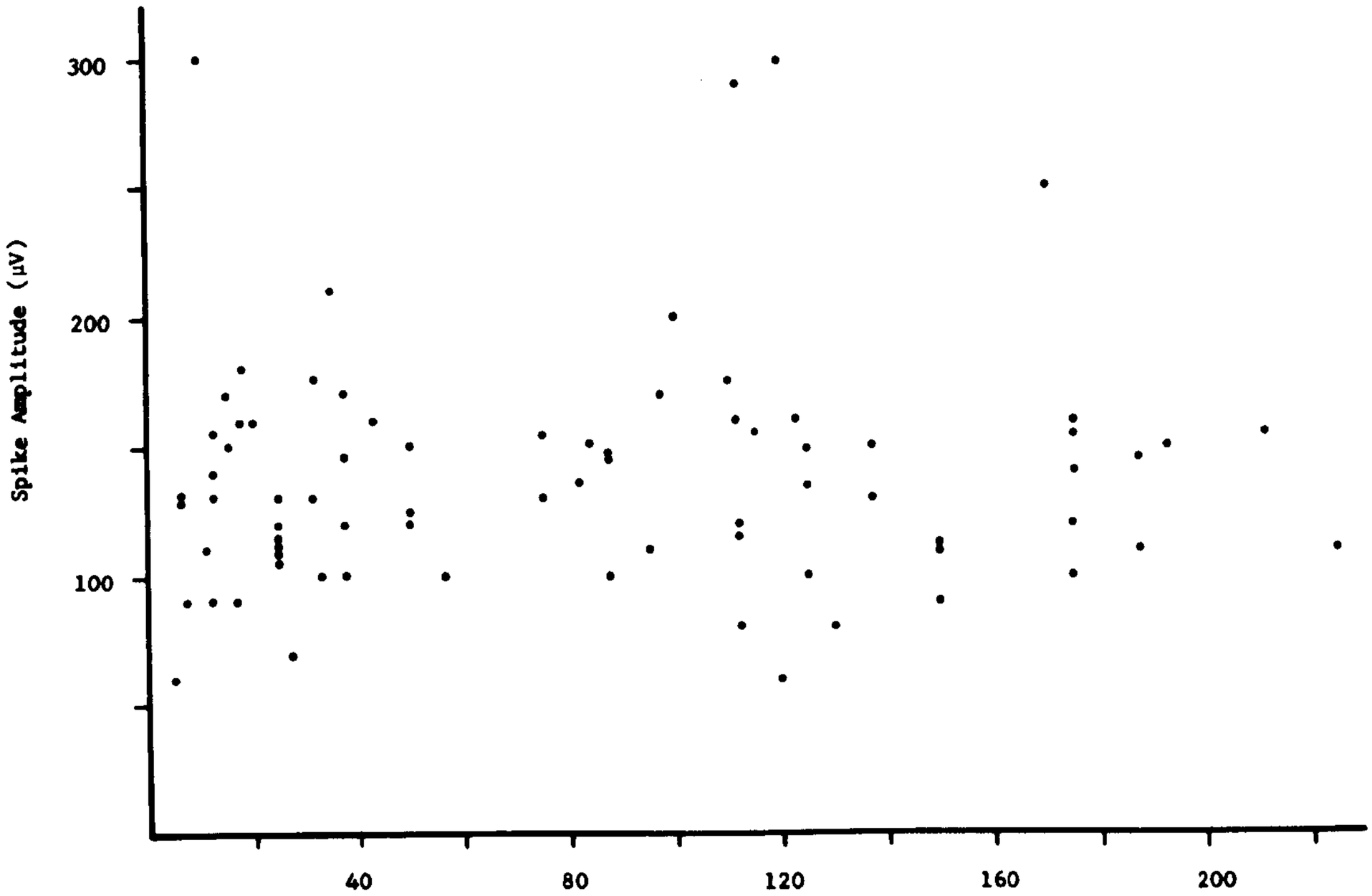


Fig. 3.9

The relationship between spike amplitude (peak to peak of extracellular action potential) and DI (velocity 4.5^{o}/sec.) for units pooled from the three jaw-closing muscles. Correlation coefficient = 0.12.

and DI and FF for pooled data. Plots of these parameters (Figs. 3.10, 3.11) failed to reveal evidence of a subdivision of units.

3.4.6 The Use of SCh in the Classification of Spindle Units

It was thought that the lack of separation of spindle units into two populations could have been due to the failure of many primary endings to show their expected dynamic sensitivity in the absence of fusimotor drive. Consequently in another series of experiments the effect of SCh was tried. This drug is believed to cause intrafusal contraction, especially of nuclear bag fibres (Smith, 1966; Boyd, personal communication), is known to excite primary endings more than secondaries (Fehr, 1965) and has been used previously to help classify spindle afferents of intermediate dynamic behaviour (Rack & Westbury, 1966).

3.4.7 Effect of SCh

Fig. 3.12 illustrates the effects of SCh on two jaw spindle units. Initially the DI of both units was similar. After SCh the resting discharge frequency rose in each case. However, one unit (A) showed a large increase in DI and in the irregularity of its firing. In contrast there was little change in the dynamic response of unit B and its discharge remained regular. These responses resemble those described by Rack & Westbury (1966) for primary and secondary endings respectively.

The instantaneous frequency of units was measured at ramp stretches of 1.0, 4.5 and 10.0°/sec of jaw opening in fourteen cats, before and after the administration of 200 μg/kg. SCh I.V. This dose was the smallest found to give consistent maximal excitation. Ramps were

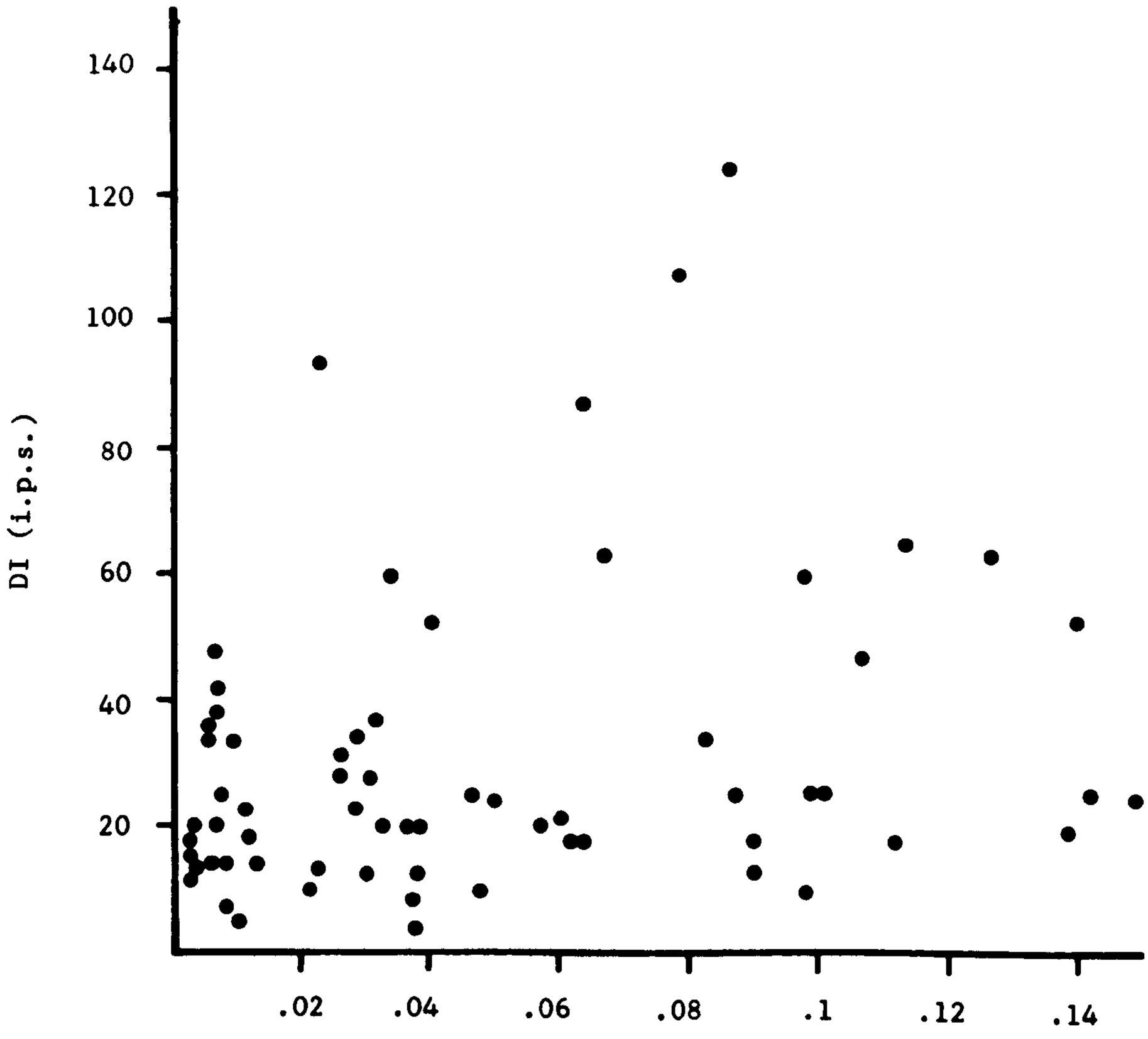


Fig. 3.10

Relationship between DI (measured at 4.5^{o}/sec.) and CV for pooled data from the three jaw-closing muscles.

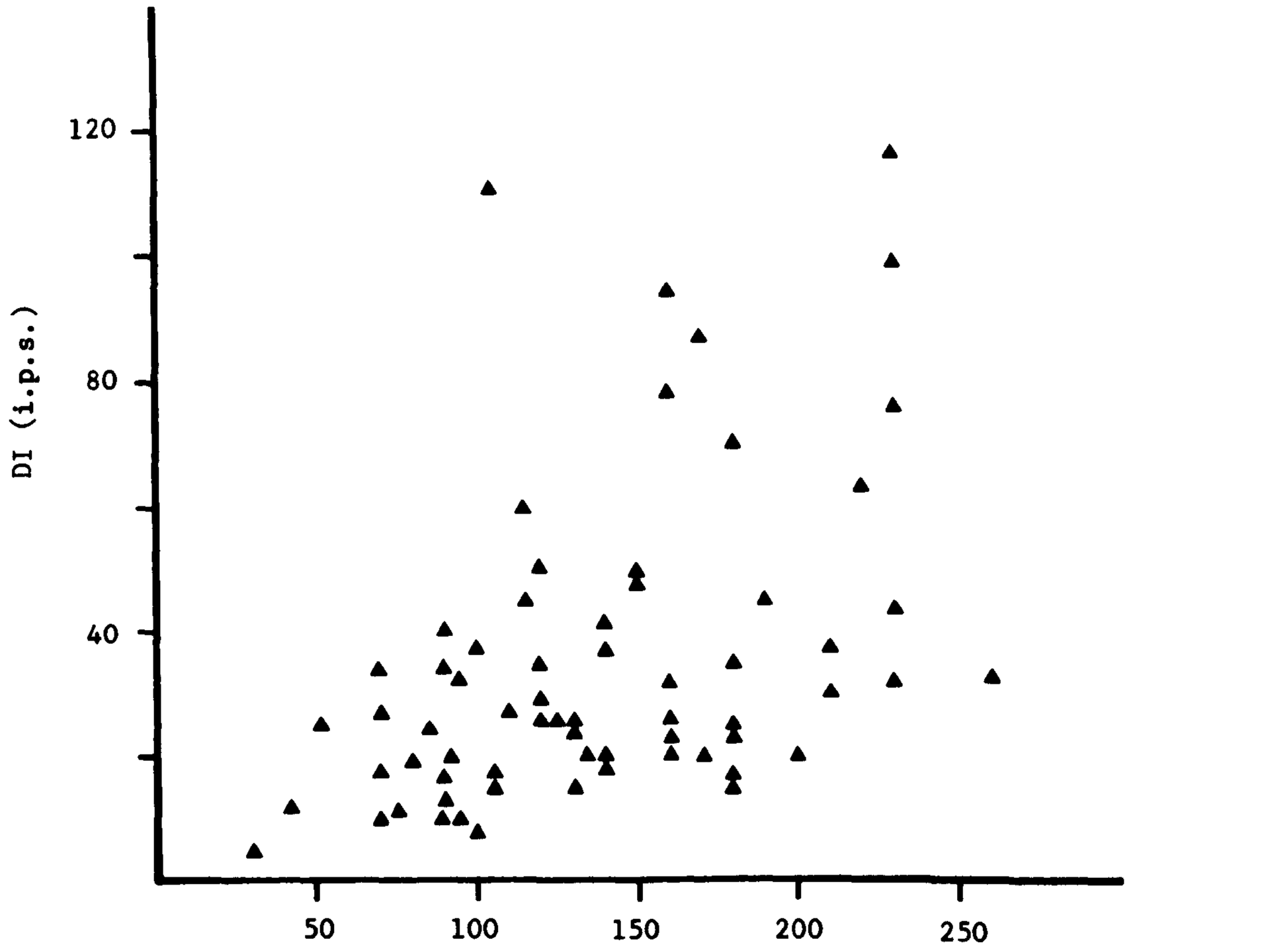


Fig. 3.11

Relationship between DI (velocity 4.5°/sec.) and FF, for data pooled from the three jaw-closing muscles.

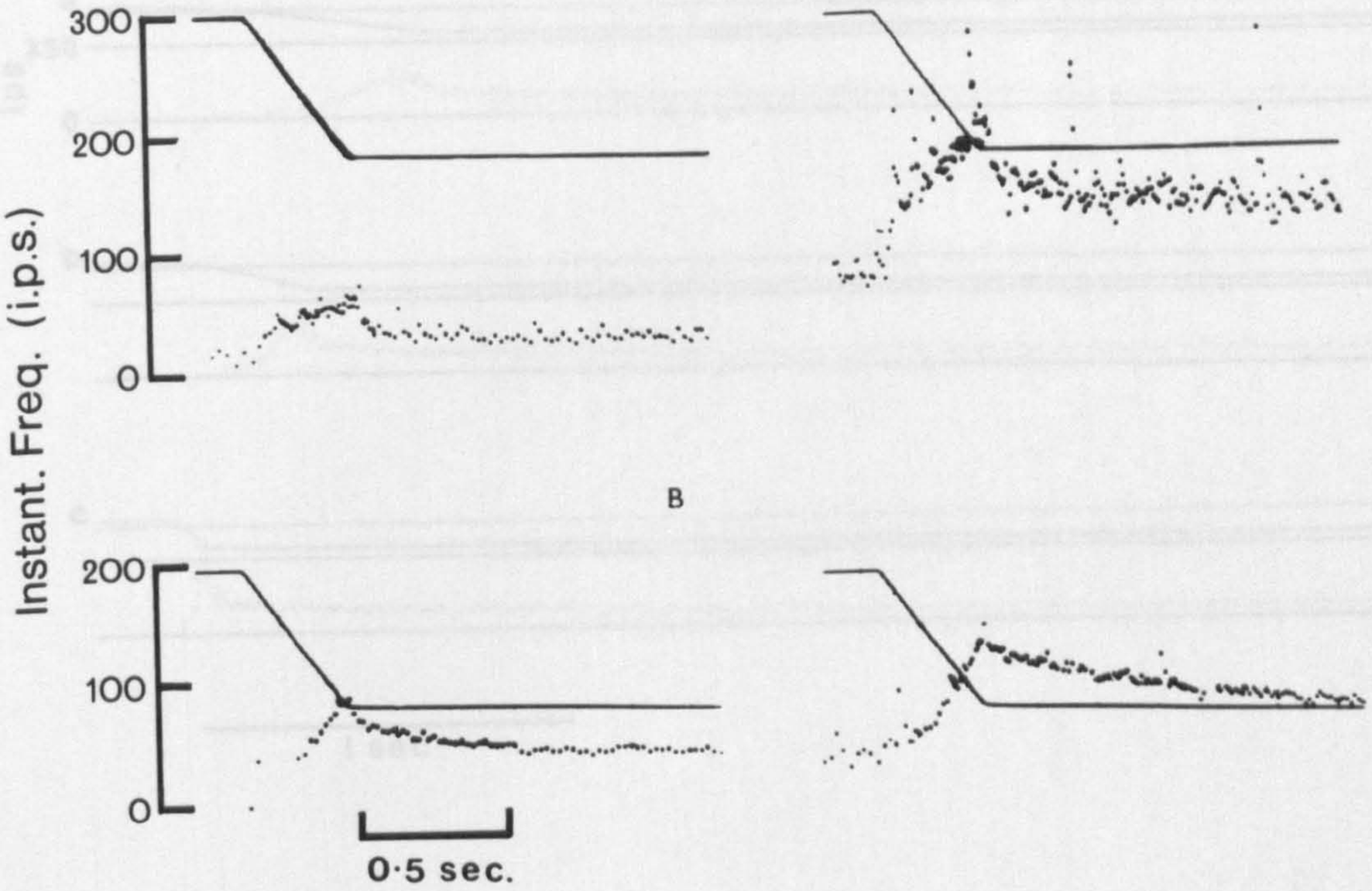


Fig. 3.12

Responses of two spindle units, (A) pterygoid and (B) temporalis to ramp jaw opening of 4.5^{o}/sec. In each case the response on the left is before,and on the right after (1 min.), the I.V. administration of 200 µg./kg. SCh.

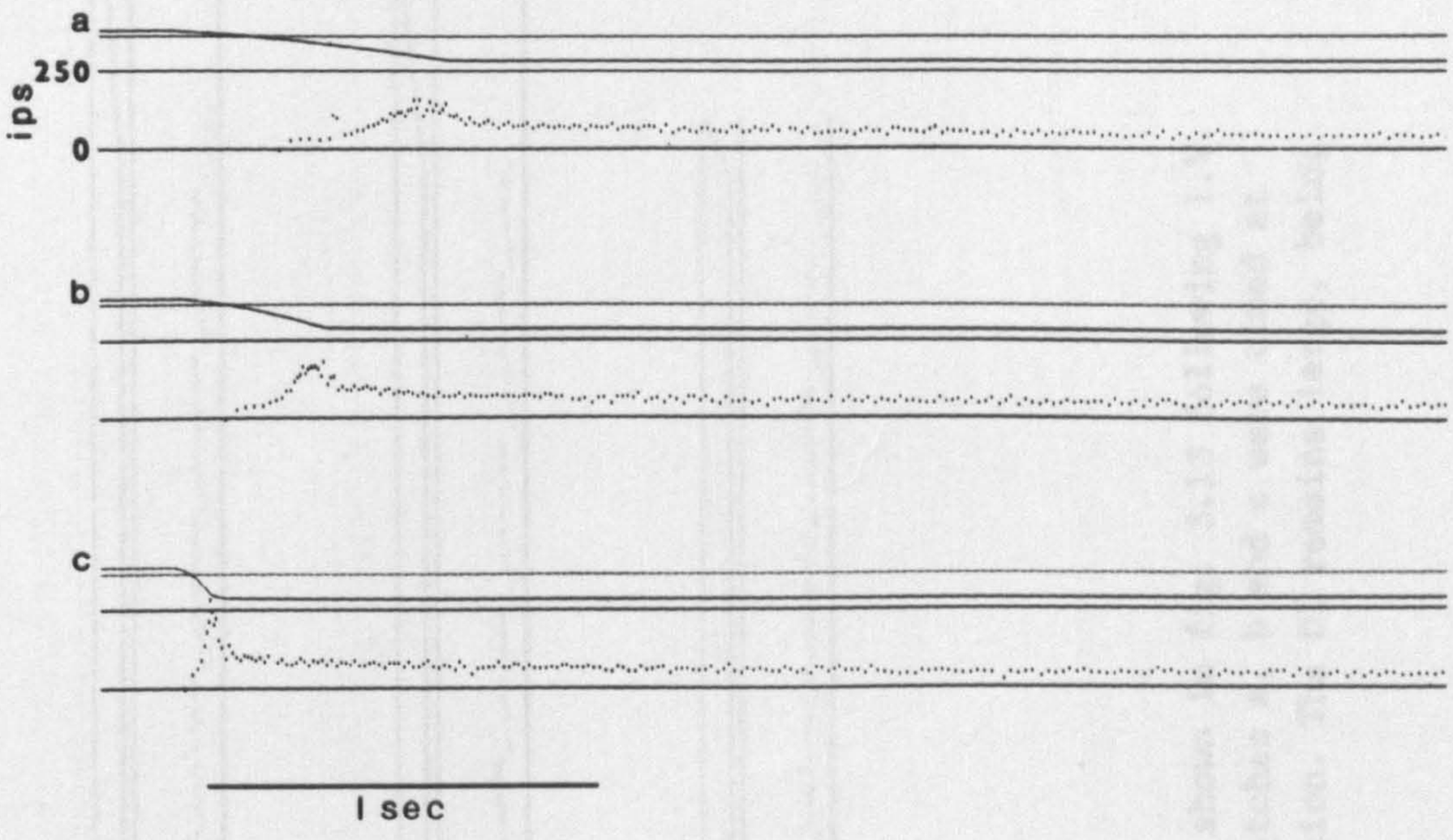


Fig. 3.13

Responses of a temporalis spindle unit to ramp jaw openings (upper trace) of a) 1.0, b) 4.5 and c) 10.0 ^{o}/sec. Discharge is shown as instantaneous frequency. The large DI indicates that the unit is probably a primary.

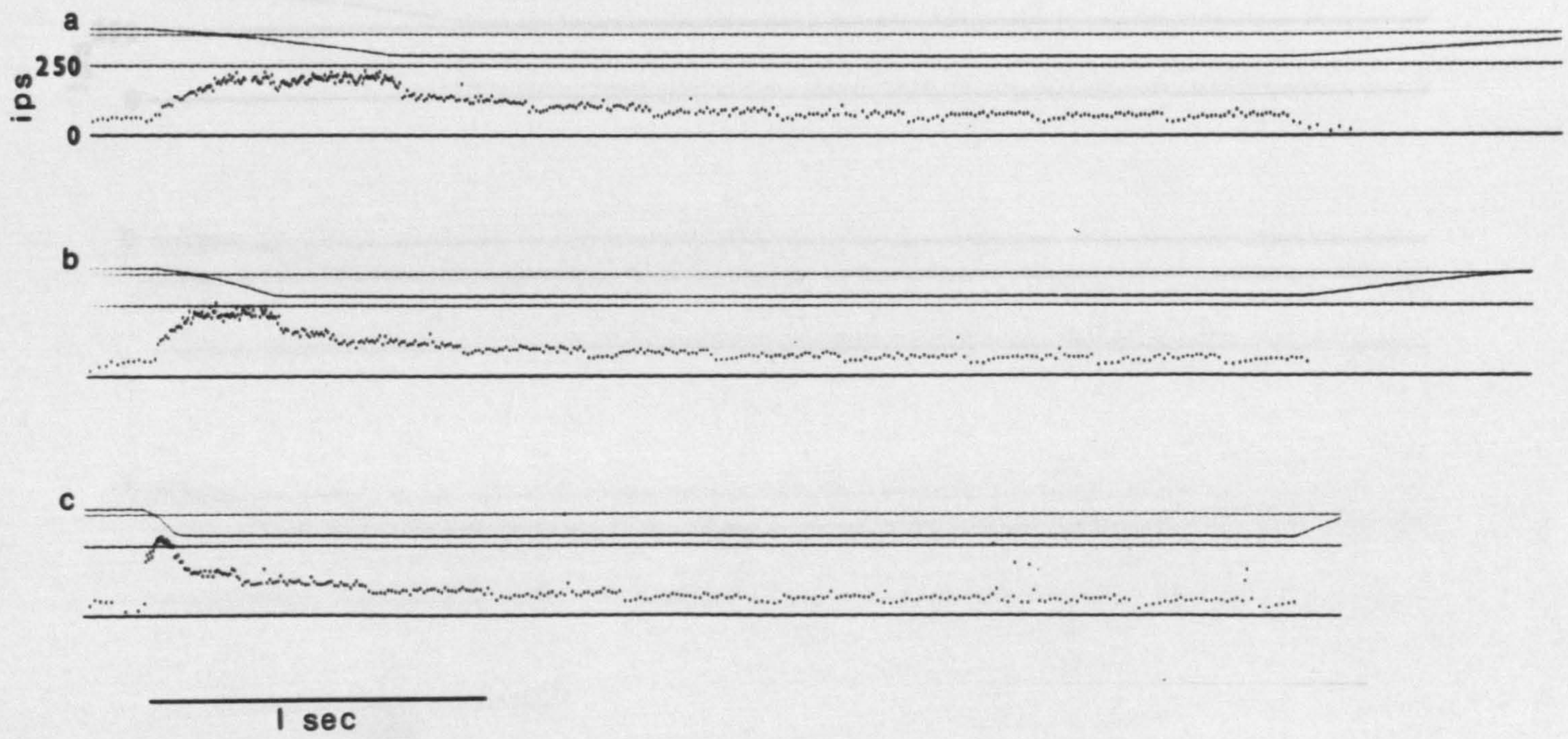


Fig. 3.14

The responses of the same spindle unit as shown in fig. 3.13 following I.V. administration of 200 µg./kg. SCh. Ramp stretches a, b and c were timed at respectively 45, 60 and 75 sec. after injection. The DI remains large, being enhanced particularly at lower velocities.

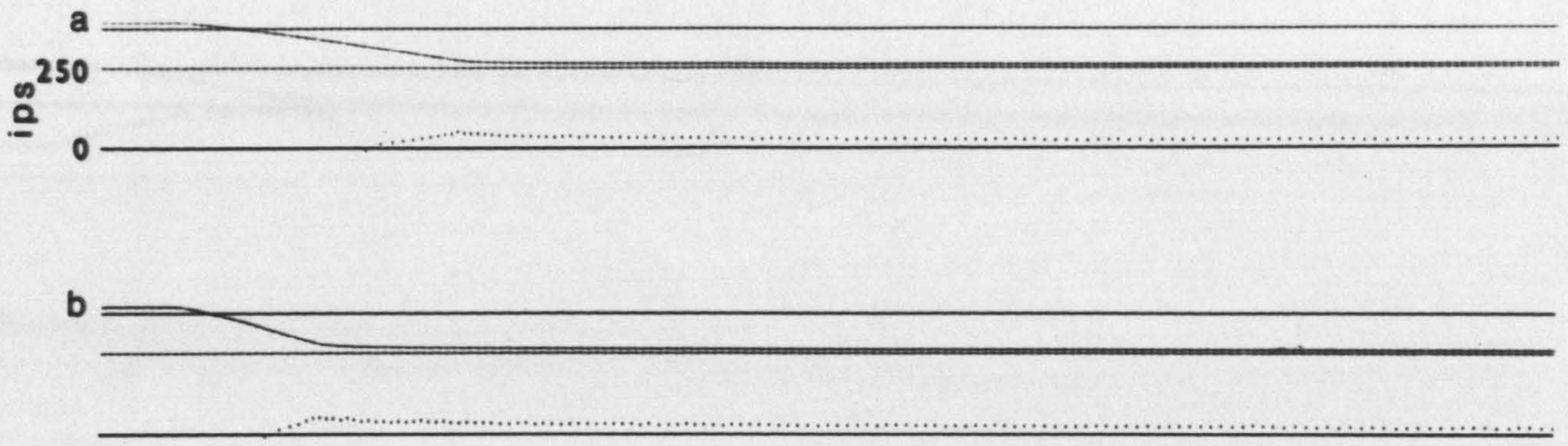


Fig. 3.15

Responses of a masseter spindle unit to ramp jaw openings (upper trace) of 1.0, 4.5 and 10.0 ^{o}/sec. Discharge is shown as instantaneous frequency. The unit has a very small dynamic response and could easily be taken for a secondary on this basis.

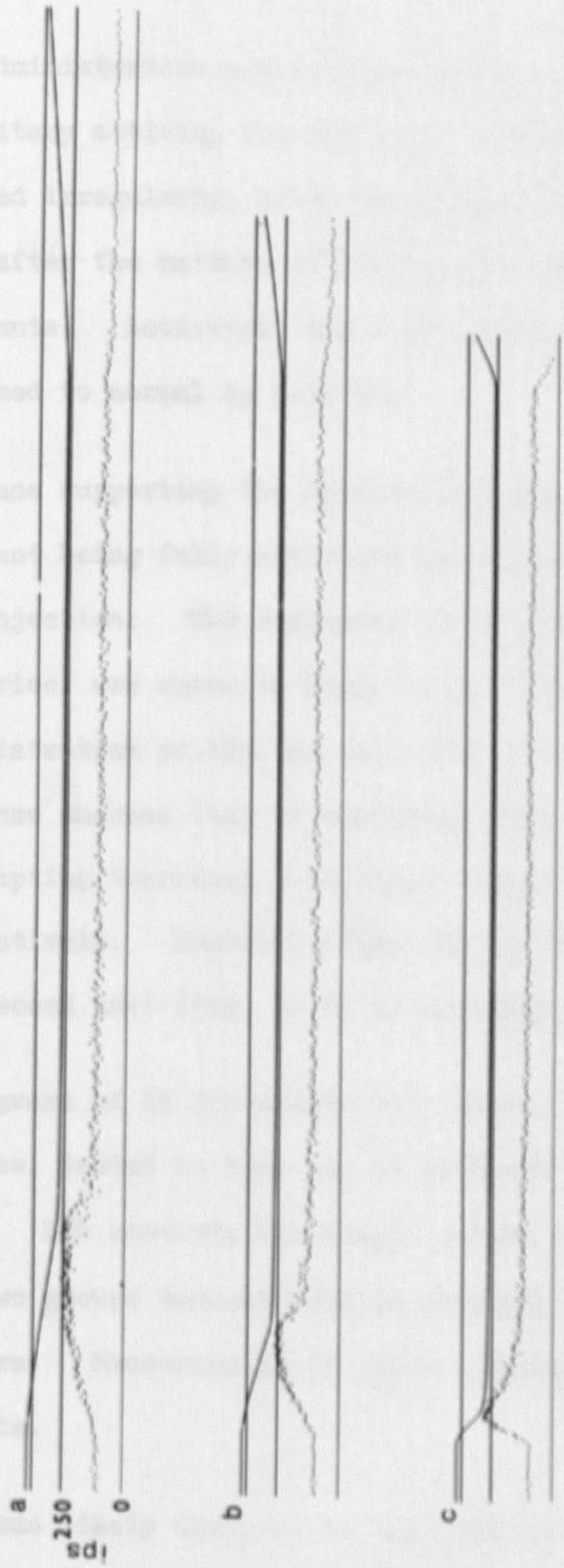


Fig. 3.16

The responses of the same spindle unit as shown in fig. 3.15 following the I.V administration of 200 mg./kg. SCh. Ramp stretches a, b and c were timed at respectively 45, 60 and 75 sec. after injection. In contrast to fig. 3.15 the spindle shows a large DI indicating that the receptor concerned is probably a primary ending.

timed at respectively 45, 60 and 75 sec. after injection. Artificial ventilation was maintained throughout.

SCh administration was followed by an initial reduction or abolition of unitary activity for 5-10 sec. Over the next 30 sec discharge increased irregularly, often being unrelated to muscle stretching. Thereafter the pattern of firing stabilized and correlated with jaw movements. Activation was most consistent at 1 min. and subsequently declined to normal by 5-10 min.

Evidence supporting the earlier proposal that many primary afferents were not being fully activated by passive stretching was seen following SCh injection. The responses of two units, both believed to be primaries, are shown in Figs. 3.13, 3.14, 3.15 and 3.16. Before administration of SCh one unit (Fig. 3.13) had a large dynamic response whereas that of the other (Fig. 3.15) was small. It would be tempting therefore to classify these units as primary and secondary respectively. However, after SCh the true dynamic responsiveness of the second unit (Fig. 3.16) is revealed.

Histograms of DI for ninety-four units, pooled from all of the jaw muscles, tested in this way at velocity 4.5°/sec. are shown in Fig. 3.17. SCh converts the single skewed distribution into a bimodal one. The two groups demonstrated in this way contain approximately equal numbers. Measurements at other stretching velocities gave similar results.

It seems likely that, as in the work of Rack & Westbury (1966), the two groups correspond to primary and secondary afferents, the former having larger DI values.

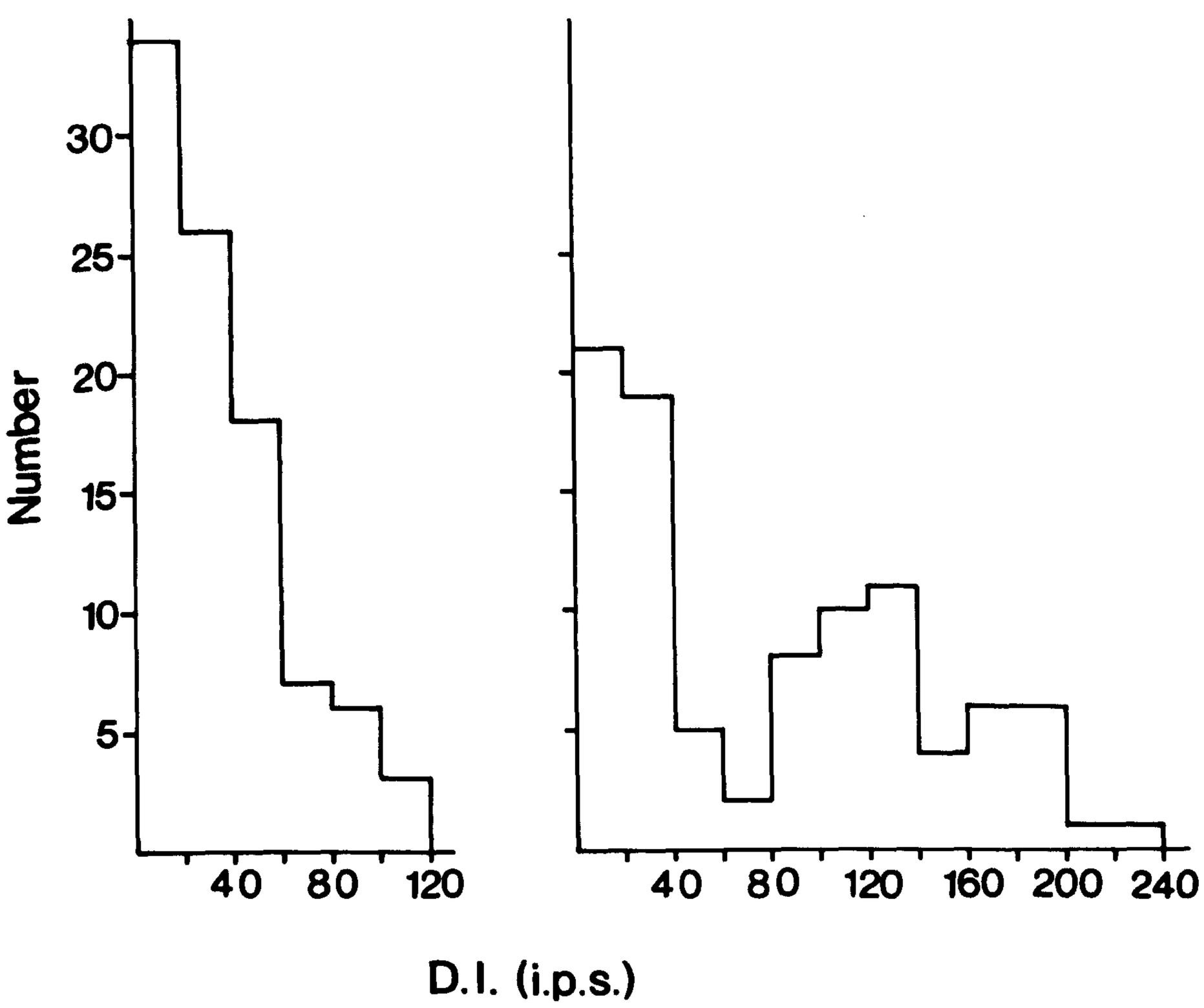


Fig. 3.17

The distribution of values of DI, measured at velocity 4.5°/sec., in ninety four spindle units (A) before and (B) after the I.V. administration of 200 µg./kg. SCh. Units pooled from the three jaw-closing muscles.

Separation of units was clear for both pterygoid and temporalis muscles, but less obvious for the masseter (Fig. 3.18). The relative numbers of units readily categorized as "primary" and "secondary" afferent cells were:- masseter 8:12, pterygoid 22:8 and temporalis 17:20.

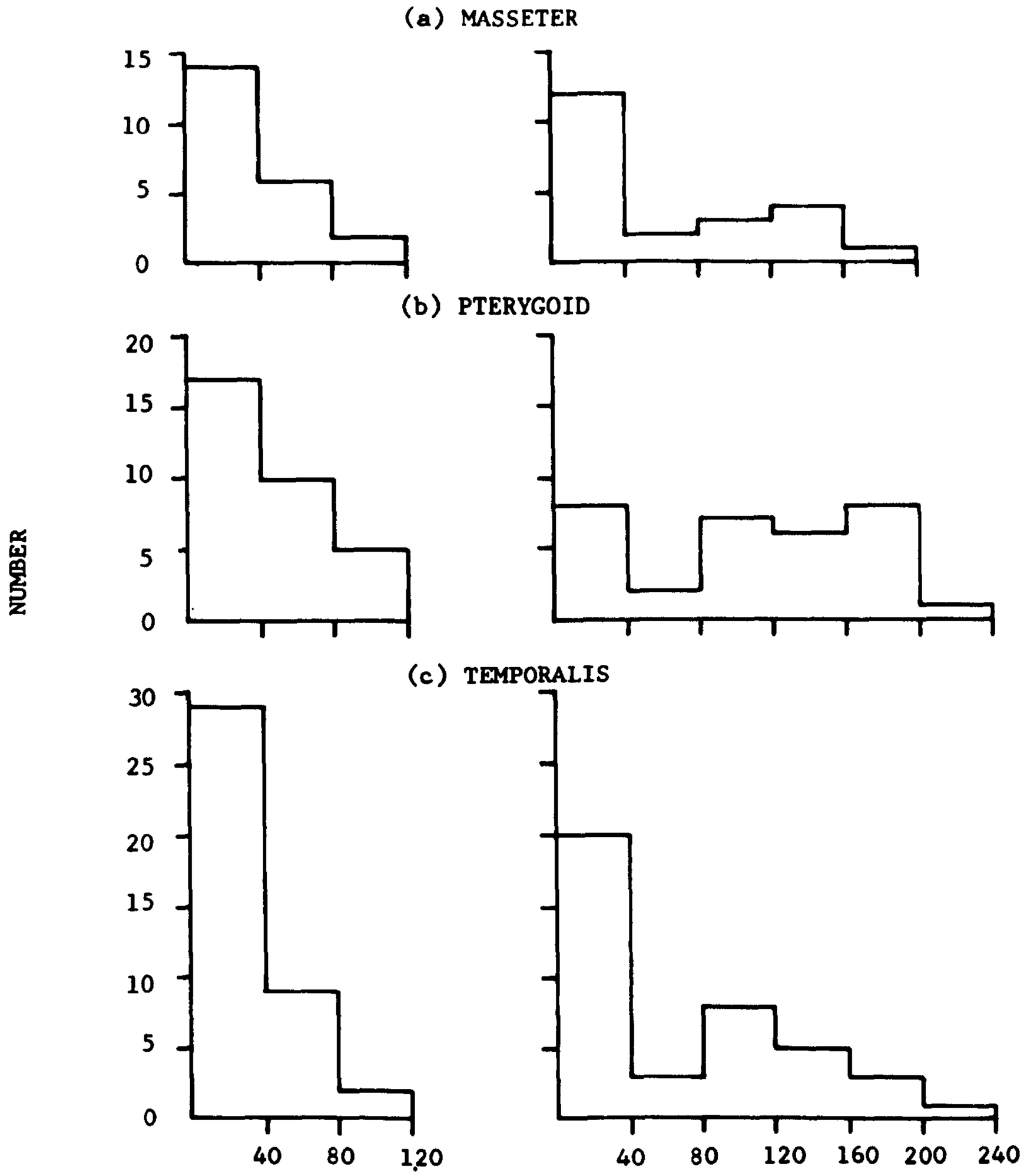


Fig. 3.18

The distribution of values of DI, measured at velocity 4.5°/sec, of spindle units in the three jaw-closing muscles (A) before and (B) after 200μg./kg. SCh. The units are those shown in fig. 3.17.

3.5 DISCUSSION

Initial efforts to classify jaw muscle spindle afferents as belonging to either primary or secondary endings, on the basis of their dynamic sensitivity to passive stretching, vibration following, discharge variability and combinations of these properties, were unsuccessful in animals whose fusimotor activity had been suppressed. Neither did the distribution of spike amplitudes of MeNV spindle units suggest such a division. Accurate conduction velocity measurements were not feasible in this preparation and in any case it is uncertain whether separation of primaries and secondaries is possible in this way for all muscles.

The apparent lack of a clear division of spindle afferents, under these conditions, implies either the absence of two functionally distinct groups of sensory ending or that the form of stimulation of receptors was inadequate.

Arguments in favour of differences in the passive transducer properties of de-efferented primaries and secondaries are largely derived from results in cat hindlimb muscles. From these findings pictures of the "typical" responses of primary and secondary endings have been built-up (see Matthews, 1972, Chpt. 4). Whilst such pictures, emphasizing differences in receptor properties, are valuable in presenting a concept that may be easily grasped, they represent an oversimplification and to this extent are misleading.

In studies on muscle spindle afferents it has become common practice initially to classify fibres, as Ia or II, according to conduction velocity and thereafter to assume that the afferent is associated with

respectively a primary or secondary ending. The usual method of sampling, i.e. splitting dorsal root filaments tends to favour the isolation of large fibres and thus to introduce bias. More important is the fact that many investigators have concentrated on fibres conducting at either well above or well below the dividing value of 72 m/sec. Fibres in the intermediate range have received far less attention.

Matthews (1963, Fig.12) in one of the first comprehensive studies shows that for cat soleus spindles, DI, at several velocities of stretching, tends to increase with the conduction velocity of afferent fibres. However his graph fails to reveal a well defined division of values of DI as might be expected from two populations of sensory ending.

In the cat tibialis anterior even greater overlap in dynamic sensitivity was found for primary and secondary endings, mainly because of a reduction in the responsiveness of primary endings (Alnaes, Jansen & Rudjord, 1965). Results from several other muscles, under comparable stretching conditions, e.g. baboon tibialis anterior (Koeze, 1968) and extraocular muscles (Bach-y-Rita & Ito, 1966) also fail to give a clear separation of dynamic sensitivities.

Furthermore in other influential papers reporting characteristic differences between primary and secondary afferents, e.g. discharge variability (Matthews & Stein, 1969) and vibration following (Brown, Engberg & Matthews, 1967) the responses of afferent fibres of intermediate conduction velocities were not analysed. In these cases the results, although showing significant differences in the behaviour of those primary and secondary endings examined, can hardly be applied with confidence to the whole population.

When fibres of intermediate conduction velocity are included it is commonly found that they show intermediate dynamic behaviour and their responses do not permit unequivocal classification (Matthews, 1963; Brown, Engberg & Matthews, 1967; Goslow, Stauffer, Nemeth & Stuart, 1973).

Another complication has been revealed by the work of Goslow, Stauffer, Nemeth & Stuart (1973) which drew attention to the marked velocity sensitivity of secondary endings of soleus and gastrocnemius under certain stretching conditions. In many previous studies on the ankle extensors of the cat the muscle was stretched 5-6 mm with the final length corresponding to the longest possible in situ length. These investigators, however, employed stretches in the "locomotor range" which although more extensive occur over a range of shorter muscle lengths.

These considerations indicate the difficulty of classifying spindle afferents in some muscles and the differences in the properties of spindles of a given muscle under different types of stretching.

Nevertheless the failure to identify two populations of receptors is not, in itself, good evidence that they do not exist.

The stretches of the jaw muscles employed in the present study, although within the physiological range, were of considerably smaller amplitude and velocity than is commonly found in eating or drinking. Also the final length of the muscle was several mm less than that attained in eating movements.

In de-efferented muscles the absence of fusimotor activity can cause "slackness" of intrafusal fibres at short muscle lengths. Indeed

Matthews (1963) found a threshold final length for some primaries to show their characteristic dynamic sensitivity. It could therefore be argued that the lack of separation of jaw spindle units was due to insufficient muscle extension. However, in a number of trials, additional stretching failed to markedly enhance the sensitivity of units and over the range normally studied the DI of spindles were linearly related to velocity.

A more probable explanation for the present observations is that the jaw muscles require appropriate fusimotor (γ_d) stimulation to show up the dynamic sensitivity of their primary endings.

Rack & Westbury (1966) used SCh to produce intrafusal contraction, especially of nuclear bag fibres, and found that under these conditions endings of previously intermediate dynamic behaviour fell into one of the two main groups.

The excitation of jaw muscle spindles in this way did lead to their division into two categories on the basis of DI. The finding of two groups of jaw spindle units, resembling primaries and secondaries in their response to passive stretching following SCh, is consistent with the histological evidence of Szentagothai (1948) in the cat, and Karlsen (1965) in the rat. Both authors described jaw muscle spindles with both primary and secondary endings.

These results stress the need for suitable fusimotor drive or pharmacologically mediated intrafusal contraction when attempting to separate primary and secondary afferents functionally. They may also explain failures in other situations.

3.6 SUMMARY

1. In deeply anaesthetized cats, in which fusimotor activity had been suppressed, attempts to separate primary and secondary jaw muscle spindle units, according to (a) DI, (b) normalized DI, (c) FF, (d) CV, (e) spike amplitude and (f) combinations of these parameters, were unsuccessful. Distributions of (a), (b), (c) and (d) were indistinguishable from lognormal.

2. Following the I.V. administration of SCh the dynamic response of many units, previously having only a small DI, was markedly enhanced. The distribution of DI was converted into a bimodal one.

3. It seems probable that the two groups of jaw spindle units, revealed in this way, correspond to primaries and secondaries respectively. Members of the former were characterized by a large DI and irregularity of discharge whereas the latter retained a low dynamic sensitivity.

4. In pooled data from the three jaw-closing muscles presumed primaries and secondaries were present in approximately equal numbers.

SECTION 4

SPINDLE BEHAVIOUR IN THE CONSCIOUS CAT

4.1 INTRODUCTION

Much information is now available on the properties of de-efferented muscle spindles as passive length detectors and of how the fusimotor system can modify their responses (Matthews, 1972). These findings have led to the formulation of a number of theories of spindle action in controlling voluntary movements.

The most widespread view is that spindles provide length(and velocity) feedback in a muscle length servomechanism. This theory was originated by Merton (1951, 1953), initially based on the "silent period" induced in a steadily contracting human thumb muscle, by a superimposed twitch, following motor nerve stimulation. The reduction in motor activity was interpreted as the response of a length servo to unexpected shortening. However this observation in itself is not good evidence that the withdrawal of spindle excitation was mainly responsible for the silencing of EMG. It is recognised that the silencing could be due to tendon organ discharge, Renshaw recurrent inhibition or resetting of motor neurone firing. Subsequent comparable experiments involving sudden release of a contracting muscle (Angel, Eppeler & Iannone, 1965) produced a similar result and were not subject to these major criticisms. These findings strongly suggest that during voluntary maintained contraction spindle activity makes a significant contribution to α-motorneurone firing.

Merton further proposed that fusimotor fibres could be responsible for supplying the command signal to a "follow-up length servo" and were thereby capable of reflexly producing contraction. Support for

the theory, in this latter form, was provided by the fact that in certain reflex motor acts, in decerebrate and anaesthetized animals, fusimotor activity could precede the onset of α-motorneurone firing (Hunt, 1951; Granit & Kaada, 1952; Eldred, Granit & Merton, 1953; Eldred & Hagbarth, 1954). It should be remembered, however, that it was clearly stated by Eldred, Granit & Merton (1953) that different movements were likely to involve varying combinations of α and γ driving.

More recently experiments in other situations have pointed to "α-γ co-activation" rather than γ leading (Granit, 1970). In respiratory muscles, in anaesthetized cats, concomitant α and fusimotor activity is such that spindle afferents show increased discharge during shortening, although contractions do not appear to be initiated solely via the fusimotor route (Sears, 1964; von Euler, 1966). Similarly in walking movements, in decerebrate cats, induced by midbrain stimulation (Severin, Orlovskii & Shik, 1967) and in reflex jaw movements in cats recovering from anaesthesia (Taylor & Davey, 1968) whilst both α and γ activity increased during contraction there was no clear evidence of the contraction having resulted from fusimotor excitation.

As a consequence of these and other experiments, described below, the theory of a"follow-up length servo" in which the input is solely via the fusimotor pathway has given way to that of servo-assistance (Matthews, 1970, 1972) with coupled α and γ inputs. Thus the concept of "α- γ " linkage popularized by Granit (1970) has gained general acceptance.

Recent human experiments have shown that during tracking, load compensation occurs with latencies consistent with the involvement of the highest parts of the CNS (Marsden, Merton & Morton, 1971) and have thereby provided indirect support for the theory proposed by Phillips (1969) that under these circumstances control may be via cortical reflexes. Such findings together with advances in techniques for cerebral recording in conscious animals (Evarts, 1966), has directed much attention away from segmental reflex mechanisms and towards higher motor centres.

This shift in emphasis is unfortunate, in some respects, since in the absence of a clear understanding of spinal processes, and especially the role of the fusimotor system, the formulation of meaningful hypotheses, regarding the whole motor control system, amenable to testing, is extremely difficult.

A principal barrier to such understanding is the lack of recordings from muscle receptors during completely normal movements in conscious animals. In both anaesthetized and decerebrate states there is considerable disturbance of both α and γ motor systems and experiments under these conditions can give only a very distorted picture of their normal operation.

Recordings are now becoming available of spindle behaviour, in human subjects, during isometric contractions of the finger muscles (Hagbarth & Vallbo, 1969; Vallbo, 1971) and certain restricted movements (Vallbo, 1973). These results emphasize the participation of the fusimotor system during voluntary contraction and lend general support to some form of "α-γ co-activation". However, as yet, these experiments have not provided convincing evidence of spindles

acting in a servo control system. Also it has not been possible to examine spindle responses during large movements in this way, because of the susceptibility of the microelectrode, sited in a peripheral muscle nerve, to displacement. Furthermore recordings appear to have been exclusively from primary spindle endings.

In view of these limitations it is desirable to extend such observations to conscious animals in which more prolonged recording during a wide range of truly "normal" movements is possible. The study of patterned movements, in particular, affords opportunities for more detailed analysis.

The jaw movements of eating and drinking in the cat are extremely stereotyped and form examples of well learned motor acts. In this situation it is to be expected that automatic load compensation be made at a low level in the motor control system.

A number of years ago Prof. A. Taylor, recognising the feasibility of recording from first order jaw-closing muscle spindle afferent cells in conscious animals, because of their location in the midbrain, started a series of experiments with this aim. Preliminary work in cats' recovery from anaesthesia (Davey & Taylor, 1967; Taylor & Davey, 1968) showed that during reflex swallowing produced by inserting fluid into the mouth, sufficient fusimotor drive accompanied movements to cause increases in spindle discharge. Evidence of fusimotor initiation of contraction was rarely seen.

In the present study experiments have been extended to the conscious animal in which jaw muscle spindle activity, jaw movements and jaw-closing muscle EMG can be recorded simultaneously during eating and

drinking. Some of the findings have been briefly reported (Cody & Taylor, 1973; Taylor & Cody, 1973; Taylor & Cody, 1974).

A similar approach has recently been applied in the monkey (Matsunami & Kubota, 1972).

4.2 METHODS

Young adult cats of either sex, chosen for their calm and friendly nature, weighing 2.5-3.5 kg. were used.

4.2.1 Surgery

Preliminary surgery was done aseptically under pentobarbitone sodium (60 mg/kg. I.P.) anaesthesia, three days prior to recording. A Perspex cylinder (1.5 cm by 1 cm diameter), for the support of a hydraulic electrode microdrive system, was centred on the stereotaxic vertical axis 3.0 mm anterior and 2.3 mm lateral, over a hole (5 mm diameter) drilled in the skull. The cylinder was securely fixed to the skull by four stainless steel screws and by acrylic cement (Surgical Simplex, North Hill Plastics Ltd.). At the time of placement of the implant the dura was left intact and an airtight cap fitted over the cylinder.

A pair of 1 mm sockets were soldered to the caudal two screws to act as the recording earth and for the attachment of a high impedance FET preamplifier.

Pairs of enamelled silver wires, with their final 4 mm bared, were inserted into the masseter and temporalis muscles for the recording of large scale EMG. These wires were brought up beneath the skin

to a 7-way microconnector (Pye, SRE7) cemented in front of the perspex cylinder.

Parallel stainless steel screws (6 mm in length, countersunk heads 4 mm diameter) were inserted into holes drilled in respectively the maxilla and mandible on one side. Each screw had a stainless steel rod set into its head so as to protrude laterally 1 cm. through the skin. Such screws were well tolerated and would remain firmly in position for several weeks.

4.2.2 Unitary Recording

Unitary recording was made with glass-coated tungsten microelectrodes Merrill & Ainsworth, 1972) of impedance 1-3MΩ at 1.7 kHz. The system used for advancing electrodes is shown in Fig. 4.1 and was made from a shortened ground glass precision syringe ("Agla", Borroughs-Wellcome). The glass piston was drilled axially and a length of hypodermic needle tubing cemented in place. Connexion was made from the upper end of the tubing to the microelectrode preamplifier. Microelectrodes were mounted slightly eccentrically in the lower end of the tubing, so that rotation of the cylinder permitted exploration of points lying on a circle of approximately 0.5 mm radius. This assembledge was connected to a second similar syringe, fitted with a microdrive, by a length of pliable nylon tubing. This closed system was filled with liquid paraffin.

Initial amplification of electrical signals recorded by the microelectrode was by the FET device (gain 100 at 1 KHz, 3 db points 190 Hz and 5 KHz) mounted on the cat's head.

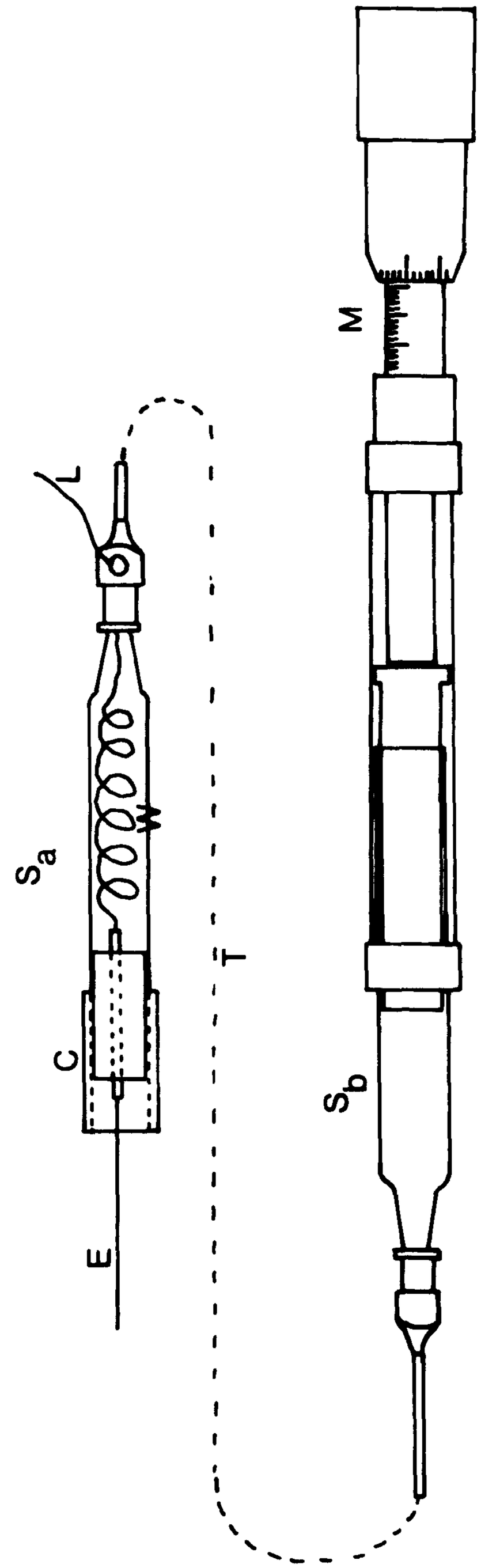


Fig. 4.1 MICROELECTRODE ADVANCER

The device consists of two precision glass syringes (S_a and S_b) joined by a length of flexible nylon tubing (T). Syringe S_b is fitted with manual microdrive (M). The closed system is filled with liquid paraffin. The electrode (E) is inserted into a piece of metal tubing cemented in the plunger of S_a. Connection to the amplifier is via a coil of copper wire (W) and a lead(L). Syringe S_a has a Perspex collar (C) which fits the cylinder implanted upon the animal's head centred over a hole centred on the MeNV.

4.2.3 EMG Recording

EMG preamplifiers (gain 200 at 1 KHz, 3 db points 150 Hz and 5 KHz) were located in a compact unit carried on a harness attached to the animal's back.

4.2.4 Jaw Movement Recording

Jaw movements were recorded by the method described by Taylor (1969). A lightweight transducer (Fig. 4.2) slipped over the rods protruding from the animal's jaws. This consisted of a V-shaped strip of spring steel, one limb of which carried a pair of silicon strain gauge elements. Short pieces of stainless steel tubing, brazed across each end of the strip, fitted over the jaw screws. The spring was sufficiently compliant (2 mm/10 gm wt. to open or close V) to produce no apparent hinderance to jaw movements. The strain gauge elements connected to the unit carried on the animal's back and thence to the remaining arms of a Wheatstone bridge circuit energized by 15.V.dc.

The recorded variables were continuously monitored on an oscilloscope (Tektronix Inc., type 565), with a loudspeaker for neural activity, and recorded on magnetic tape (Thermionic TF4000, F.M. bandwidth 0-2.5 KHz).

Fig. 4.3 diagramatically illustrates the main features of the various recording systems.

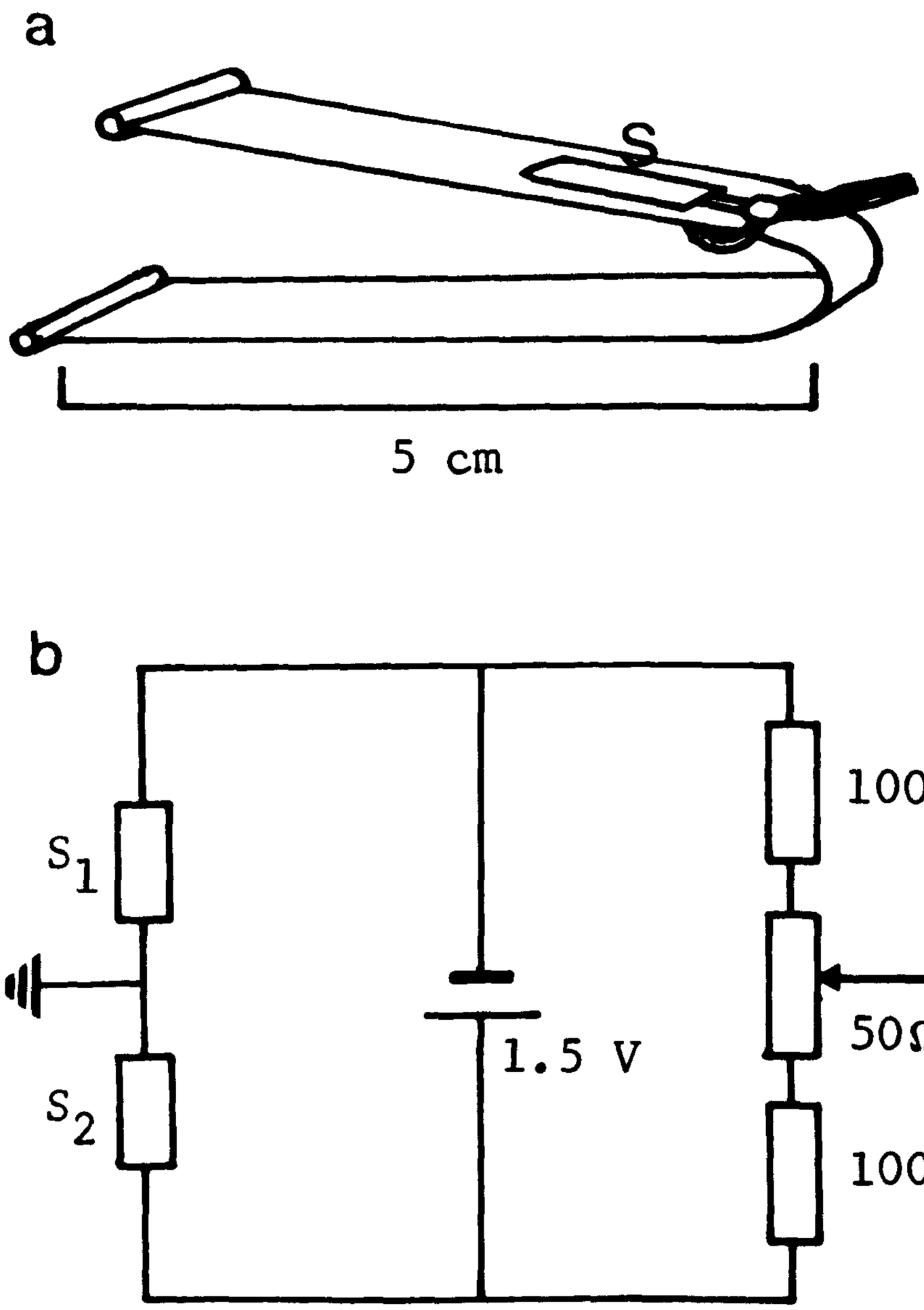


Fig. 4.2

(a) Jaw movement transducer.

(b) Strain gauge elements (S_1 and S_2) are connected as two arms of a Wheatstone bridge.

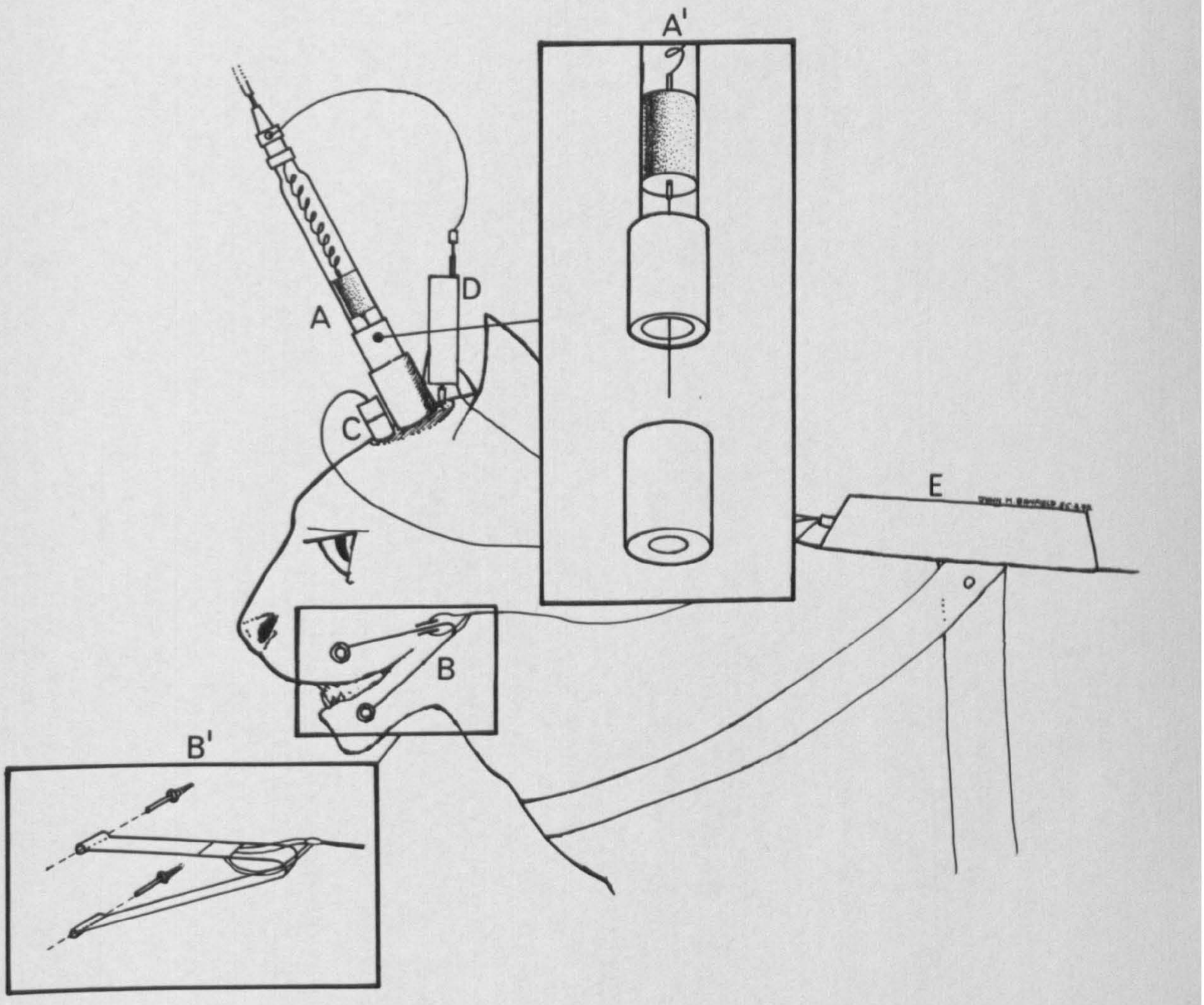


Fig. 4.3

Features of the recording apparatus. A (inset A^1) microelectrode advancer.
B (inset B^1) jaw movement transducer.
C EMG microconnector
D FET preamplifier.

4.2.5 Identification of Jaw-Closing Muscle Spindle Units

Cells were identified as jaw-closing muscle spindle units according to the following criteria.

(1) Stereotaxic location. Attention was restricted to units with stereotaxic coordinates corresponding to those of the MeNV. During vertical electrode penetrations the surface of the superior colliculus provided a useful depth reference. This was easily recognized by the onset of visually related activity. The MeNV is known to be situated some 5 mm deeper.

(2) Discharge pattern. The activity of presumed spindle units was closely related to jaw movement.

(3) Effects of local muscle pressure. Marked increases in discharge of "high frequency" units (presumed primaries) could typically be elicited by discrete surface pressure on one of the jaw-closing muscles. "Low frequency" units (presumed secondaries) were far less sensitive to probing and confident identification of the muscle of origin was not always possible.

(4) Passive muscle stretch. In several instances the animal would tolerate passive manipulation of the jaw. The usual response was a considerable speeding of unitary firing during opening. However, this was variable, even in the same unit at different times, suggesting that the response was very dependent on the existing state of fusimotor activation.

4.3 RESULTS

4.3.1 Patterns of Jaw Movement and EMG Activity in the Jaw-Closing Muscles During Eating and Drinking

In the cat, jaw movements are simple, consisting of essentially hinge-like opening and closing, with little lateral deviation.

During both eating and drinking, animals made a series of repeating patterned movements. These were especially stereotyped in lapping.

When eating tinned cat meat successive movements of 25-30° of jaw displacement were made. Jaw angle, together with smooth rectified EMG (Sre) from the two principal jaw closers, masseter and temporalis, is shown in Fig. 4.4. Each cyclic movement typically occurred in four main phases:-

(1) a large rapid closure (approx. 20° in 50 msec), (2) a smaller more gradual closure (approx. 10° in 120 msec), (3) a slow partial opening (approx. 10° in 100 msec) and (4) a rapid final opening (approx. 20° in 80-90 msec).

Such cycles were repeated at about 3 per sec.

Patterns of simultaneously recorded EMG activity varied somewhat between preparations, presumably due to differences in electrode placement and recording surface area.

In many cases both masseter and temporalis activity was closely related to the closing phase, its appearance coinciding with or slightly preceding jaw movement, and persisted until 80-90% of closing had been achieved (Fig. 4.4). Consistently temporalis EMG was of greater amplitude.

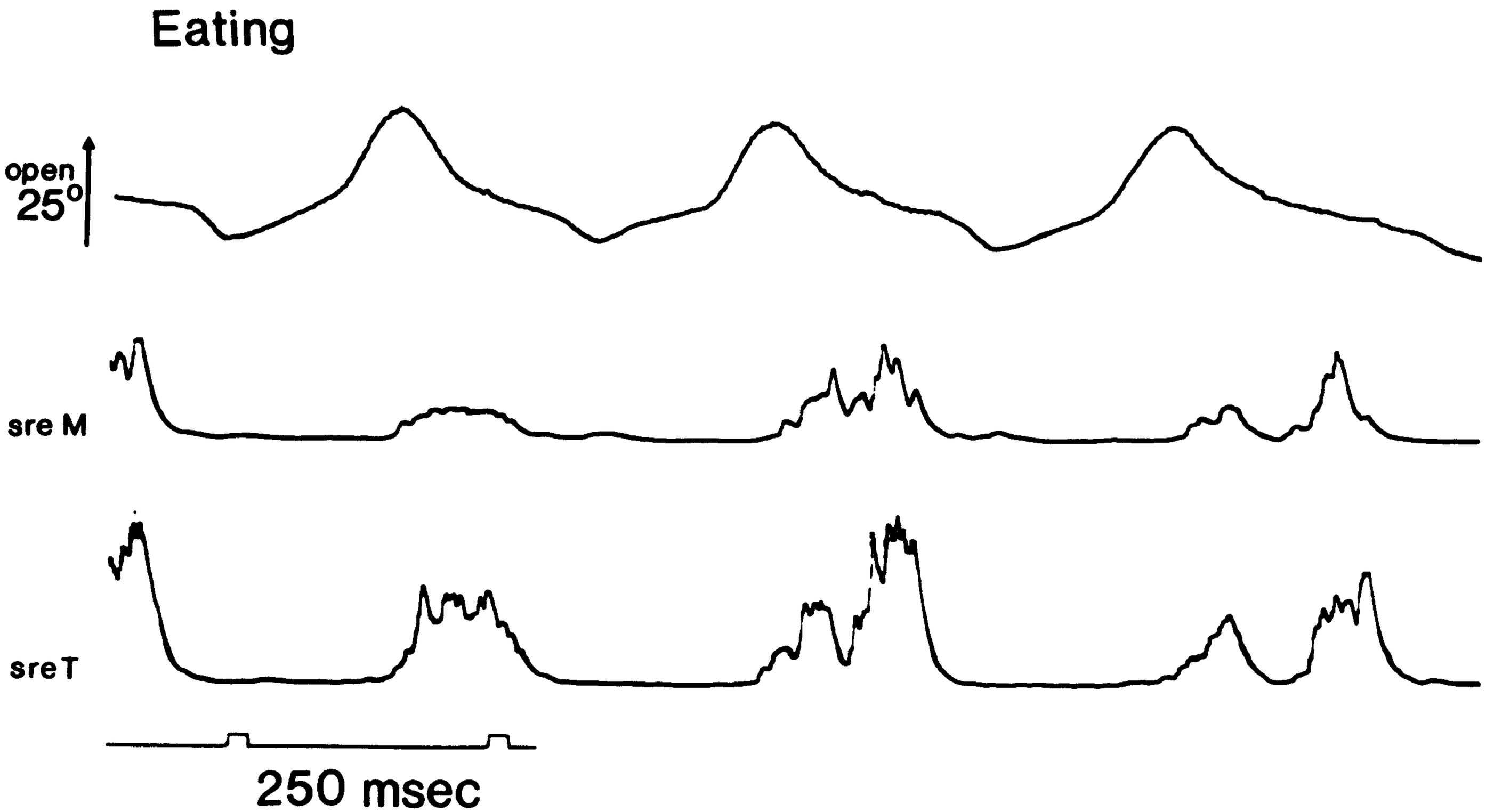


Fig. 4.4

The relationship between jaw movements and EMG in the jaw-closing muscles during eating. Upper trace represents jaw displacement. Middle and lower traces show smoothed rectified EMG from masseter (sreM) and temporalis (sreT). EMG was smoothed with a time constant of 10 msec.

Often, however, the form of masseter EMG was more complex (Fig. 4.5). The main EMG bursts preceded those of temporalis, by some 50 msec, and finished earlier. Also additional smaller bursts were seen, between the main ones, corresponding to the completion of the closing movement and commonly continuing into the early part of opening. This intermediate activity may be related either to swallowing or to compression of the cheeks.

Lapping involved a series of small rapid movements of 7.5 - 10°, punctuated after every four or five by a swallowing manoeuvre. Fig. 4.6 shows such a series. The sequence of jaw movements differed from those of eating in that opening took place in a single continuous motion and that the second closing phase was far faster. Again, EMG activity from the jaw-closers occurred principally during closing. The exception was that during a swallowing movement a burst of masseter EMG occurred during opening, suggesting that co-contraction of this muscle and jaw-opening muscles must have been taking place.

4.3.2 General Pattern of Spindle Behaviour During Jaw Movements

Recordings have been made from twenty-one spindle units in eleven cats.

The most striking feature of spindle unitary responses was that, during eating and drinking, they were of the general form expected during passive movements. Maximal firing occurred during opening, when the muscles were being stretched. During the shortening phase spindle discharge was normally reduced or silenced. This can be clearly seen in Fig. 4.7 which shows a multi-spindle unit record during eating. Conspicuous silencing is seen during several of the closing phases, and in all cases firing is reduced at this time.

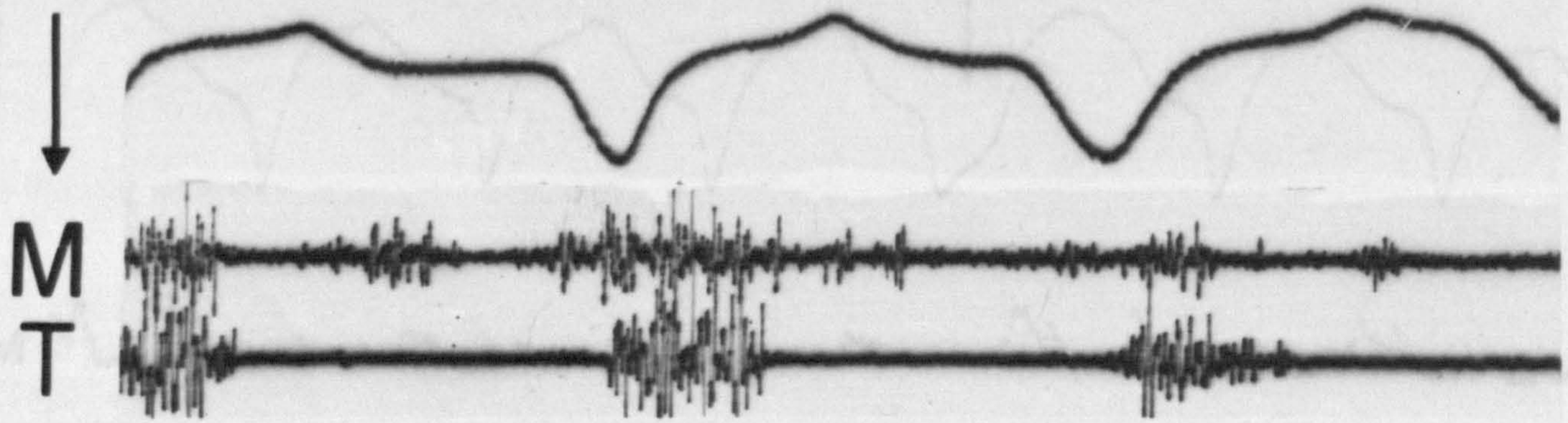


Fig. 4.5
Intermediate bursts of masseter EMG (M) during eating. Arrow: 25° opening. Bar: 0.5 sec

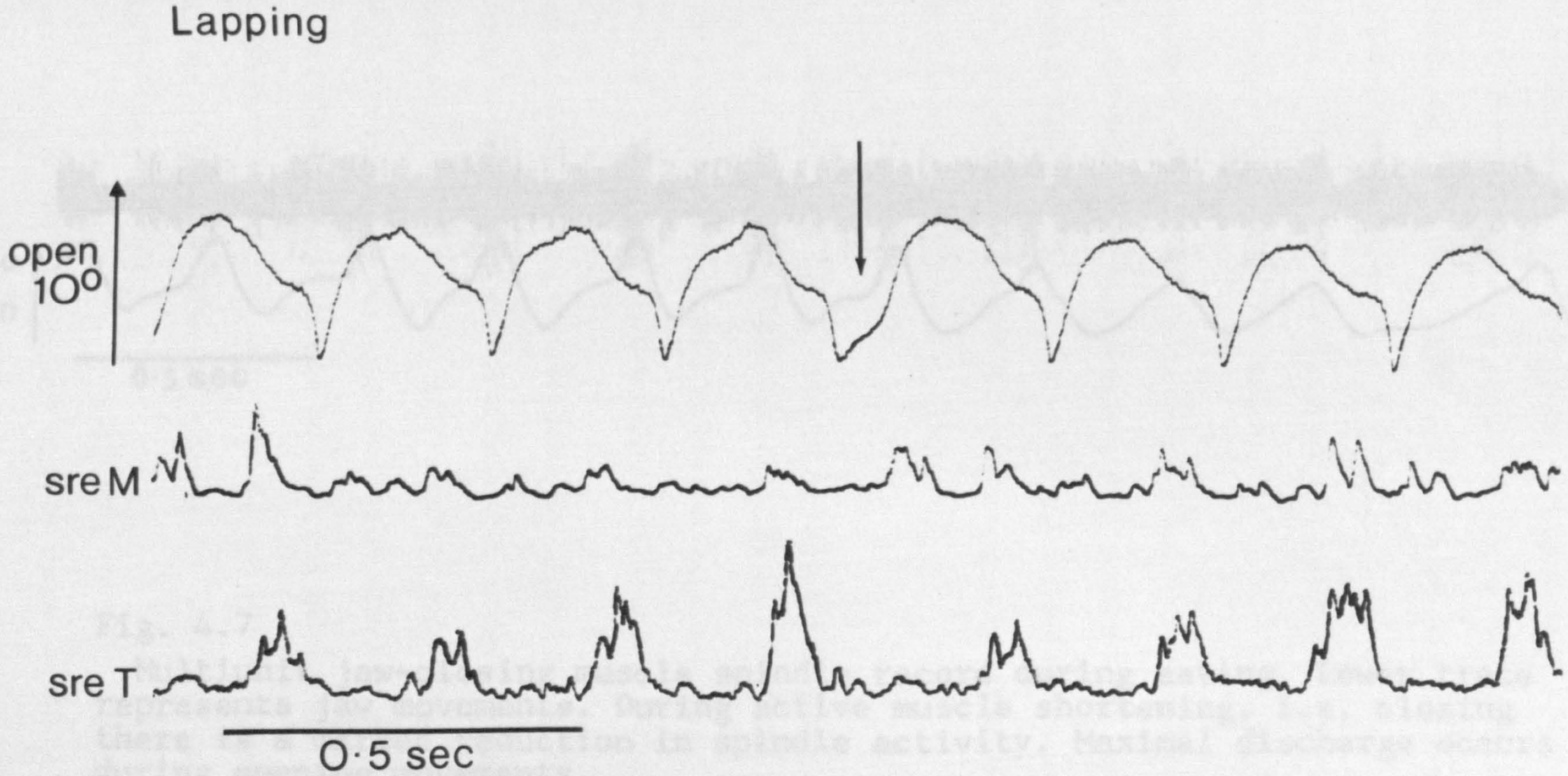


Fig. 4.6

The relationship between jaw movement and smoothed rectified EMG (time const. 10 msec) during lapping. The central arrow indicates a swallowing movement.

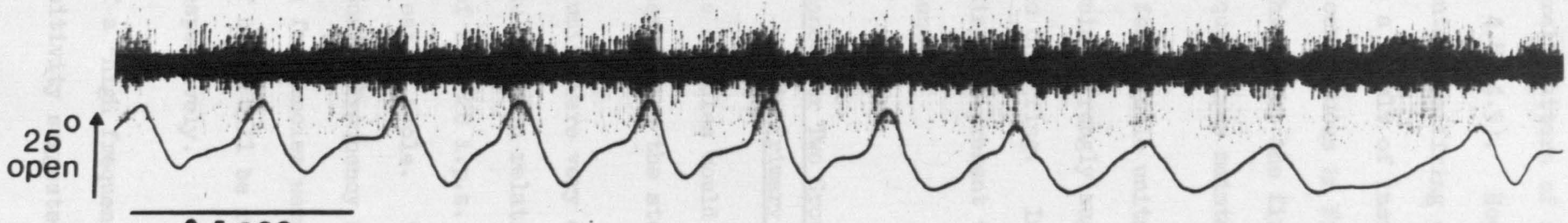


Fig. 4.7

Multiunit jaw-closing muscle spindle record during eating. Lower trace represents jaw movements. During active muscle shortening, i.e. closing there is a marked reduction in spindle activity. Maximal discharge occurs during opening movements.

A similar overall pattern of activity may be seen in single unit records (Fig. 4.8, 4.9). However, during several of these large eating movements some firing continues during the shortening phase, presumably as a result of increased fusimotor drive. This is particularly conspicuous in Fig. 4.9b, when the animal was licking its lips. Throughout the first two movements a remarkably steady discharge frequency was maintained.

The tendency for spindle units to continue to fire despite extensive muscle shortening strongly suggests that concomittant fusimotor activation was occurring. Indeed in some cases sufficiently close α - γ matching was present to avoid appreciable fluctuations in spindle frequency.

4.3.3 Evidence for Two Groups of Spindle Units Corresponding to Primary and Secondary Afferents

Cat jaw muscle spindles could be divided into two groups according to their responses during the stereotyped movements of eating and drinking.

One group of units were very dynamically sensitive, showing marked frequency modulation in relation to length changes. Maximal firing frequencies of 220-320 i.p.s. were obtained during the lengthening phase of the eating cycle. The remainder were far more static in their behaviour. Frequency modulation was less marked and maximal instantaneous frequencies were lower (range 60-160 i.p.s.). These two groups of units will be referred to as "high frequency" and "low frequency" respectively.

An example of a "high frequency" unit is shown in Fig. 4.10. Local pressure sensitivity suggested that the ending was located in the

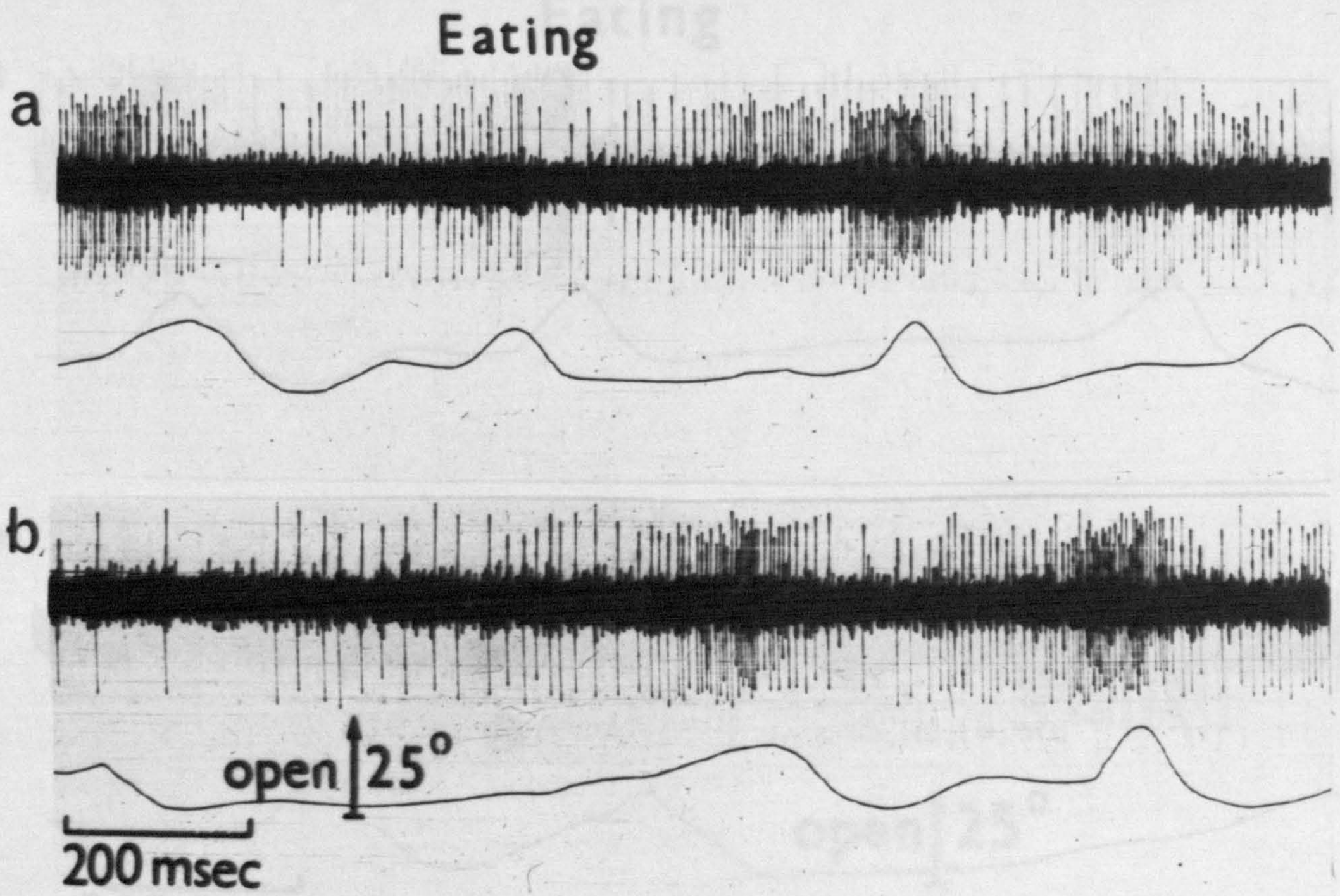


Fig. 4.8

Responses of a jaw-closing muscle spindle afferent unit during eating. Lower trace represents jaw movements. Unit believed to be from temoralis.

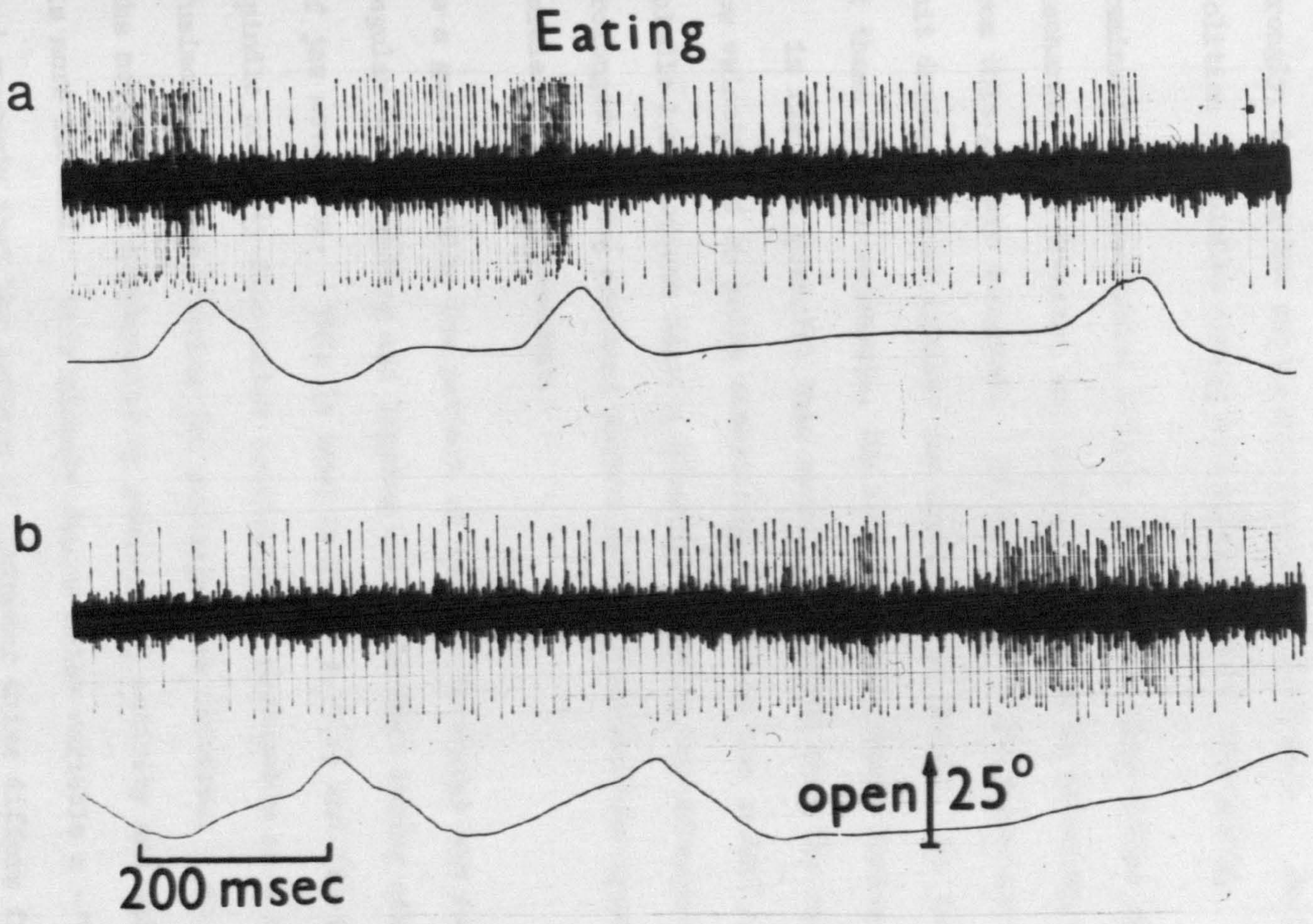


Fig. 4.9

The same unit as in fig. 4.8. In (b) the animal was licking its lips and large movements were accompanied by little change in spindle frequency.

temporalis muscle. The unit responds primarily to the velocity of jaw movements during eating. During the initial slow lengthening phase the spindle responded with a high frequency burst. As velocity decreased the spindle was silenced, only to be excited, even more strongly, during the subsequent more rapid extension. Prolonged abolition of spindle firing accompanied active shortening.

Examination of successive eating cycles shows that whilst spindle discharge, when present, was generally related to velocity, variations from this pattern occurred. In Fig. 4.10(b) the behaviour of this unit during several smaller jaw movements is shown. In the first of these smaller movements, the second spindle burst during extension is far more powerful than would be expected from the relatively low velocity of on-going stretching, whilst in the second a comparable spindle burst occurs despite closing. During the subsequent prolonged opening movement marked spindle fluctuations appear to be unrelated to displacement.

As a generalization the pattern of spindle discharge was far more regular during eating and lapping movements than during other types of jaw movements. This is seen in Figs. 4.11(a) and (b) in which spindle activity fluctuates considerably, presumably as a result of fusimotor changes, during two prolonged jaw closures. In Fig. 4.11(a) the movement is accompanied by greater EMG activity and spindle firing is more reduced. This clearly demonstrates variable α - γ activation, and suggests that the pattern of fusimotor drive differs from that present during repetitive eating movements.

Further examples of high frequency unit activity are shown in Figs. 4.12, 4.13(a), 4.14(a).

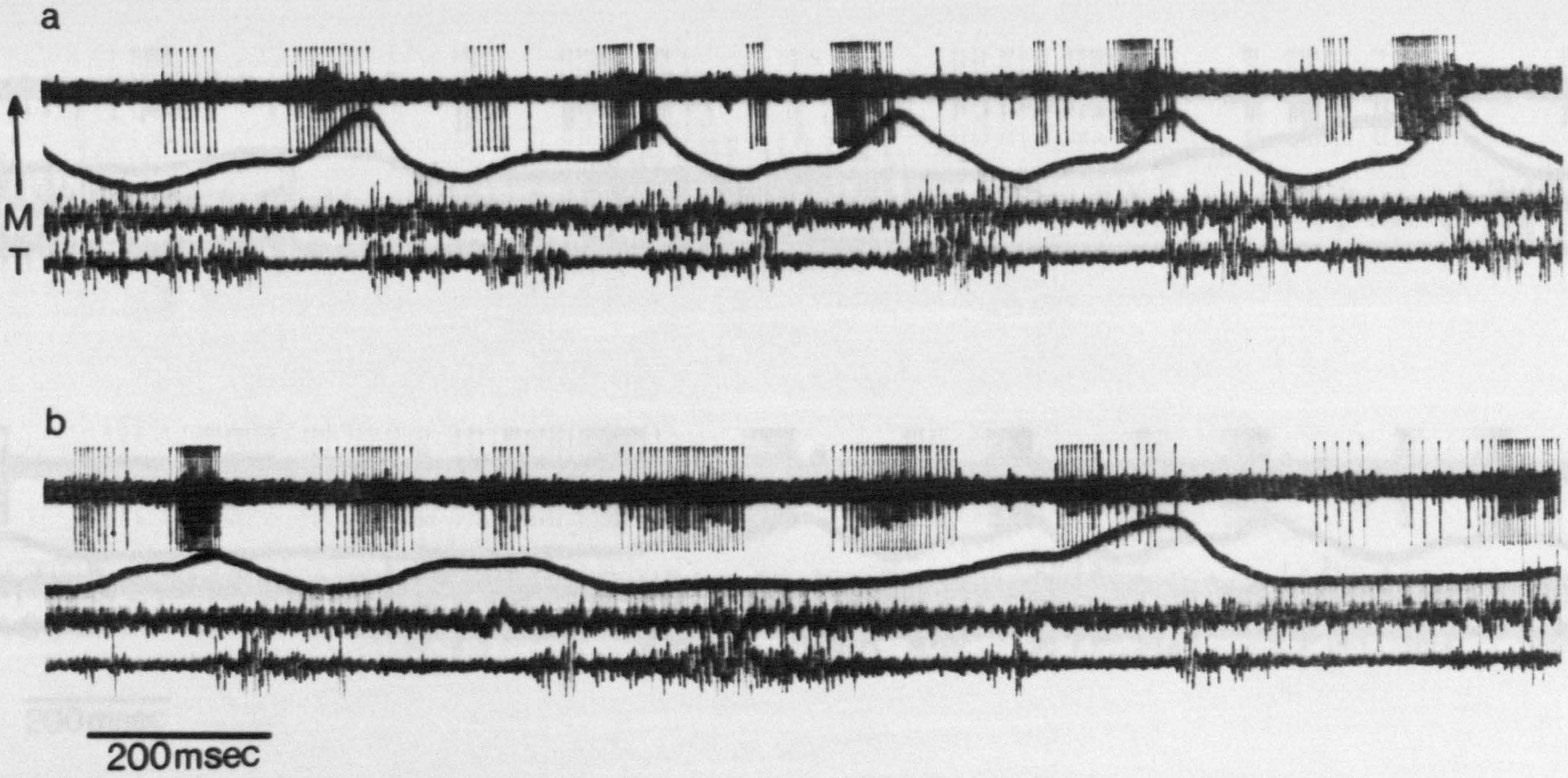


Fig. 4.10

Responses of a "high frequency" temporalis unit during eating. The arrow represents 25° of jaw opening. Masseter and temporalis EMG are labelled (M) and (T) respectively.

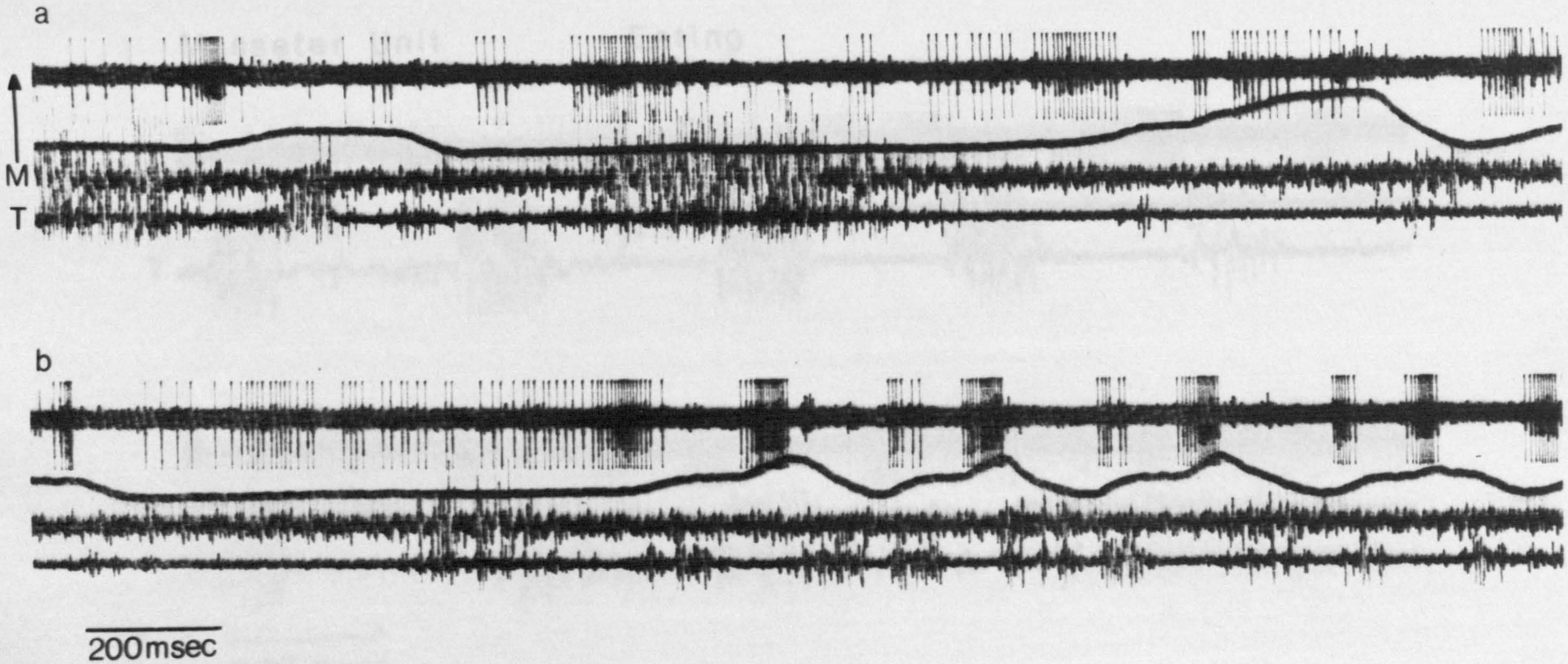


Fig. 4.11

Activity of the same unit as shown in fig. 4.10 during prolonged closing movements. The fluctuation of spindle disharge suggests variable fusimotor excitation.

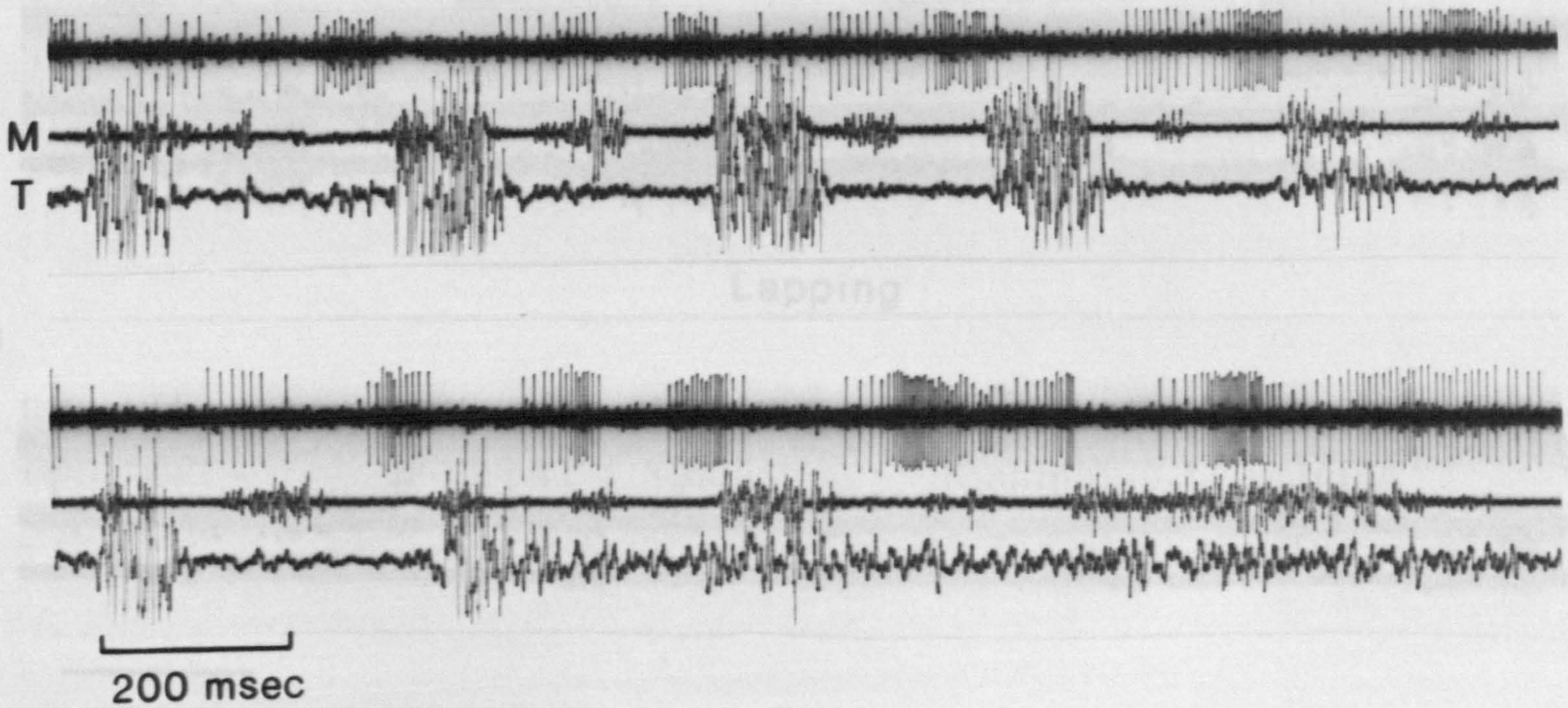


Fig. 4.12

Responses of a "high frequency" masseter unit, together with EMG from the masseter (M) and temporalis (T) muscles during eating.

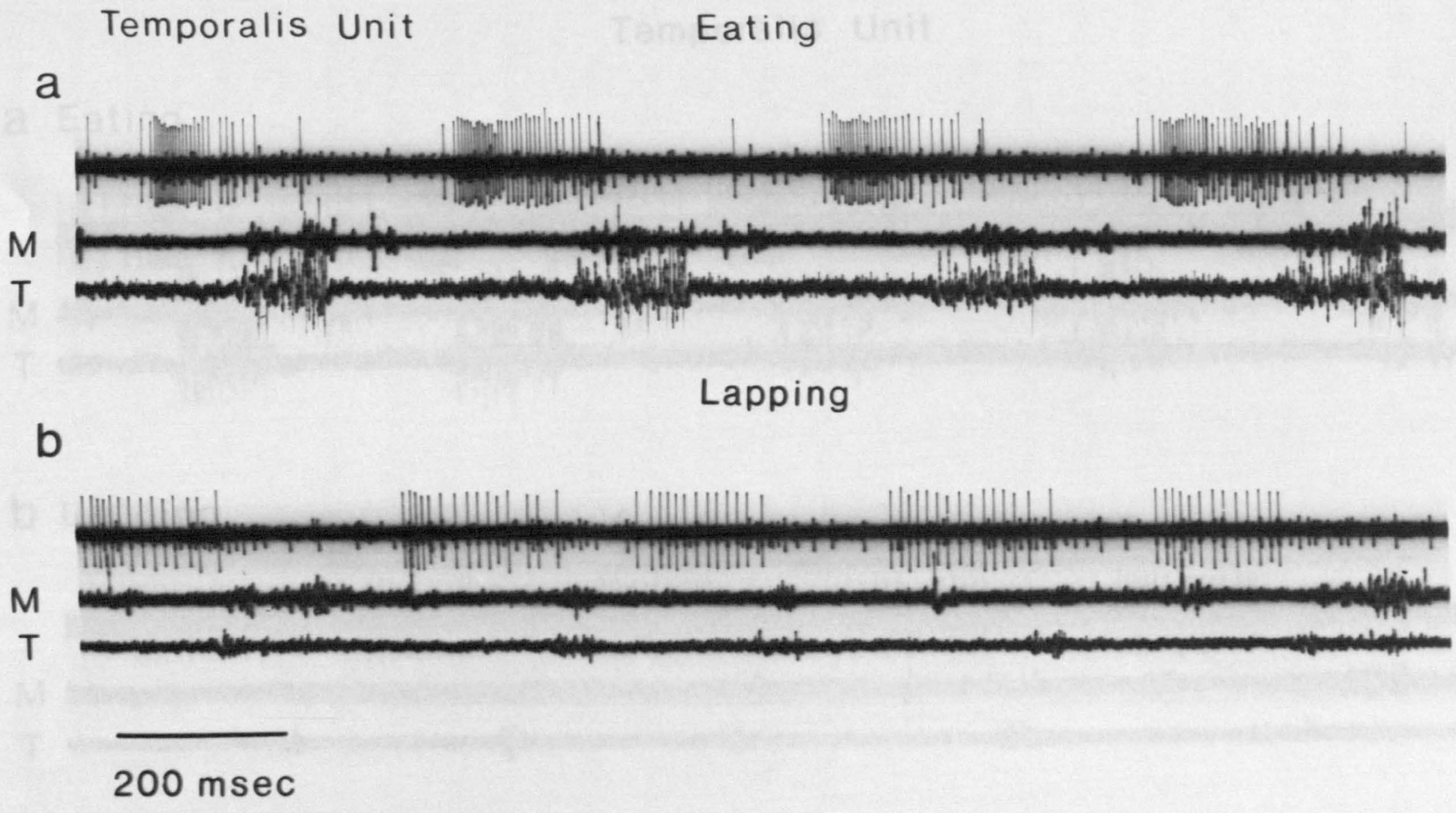


Fig. 4.13

Responses of a "high frequency" temporalis unit, together with EMG from masseter (M) and temporalis (T) muscles, during eating and lapping.

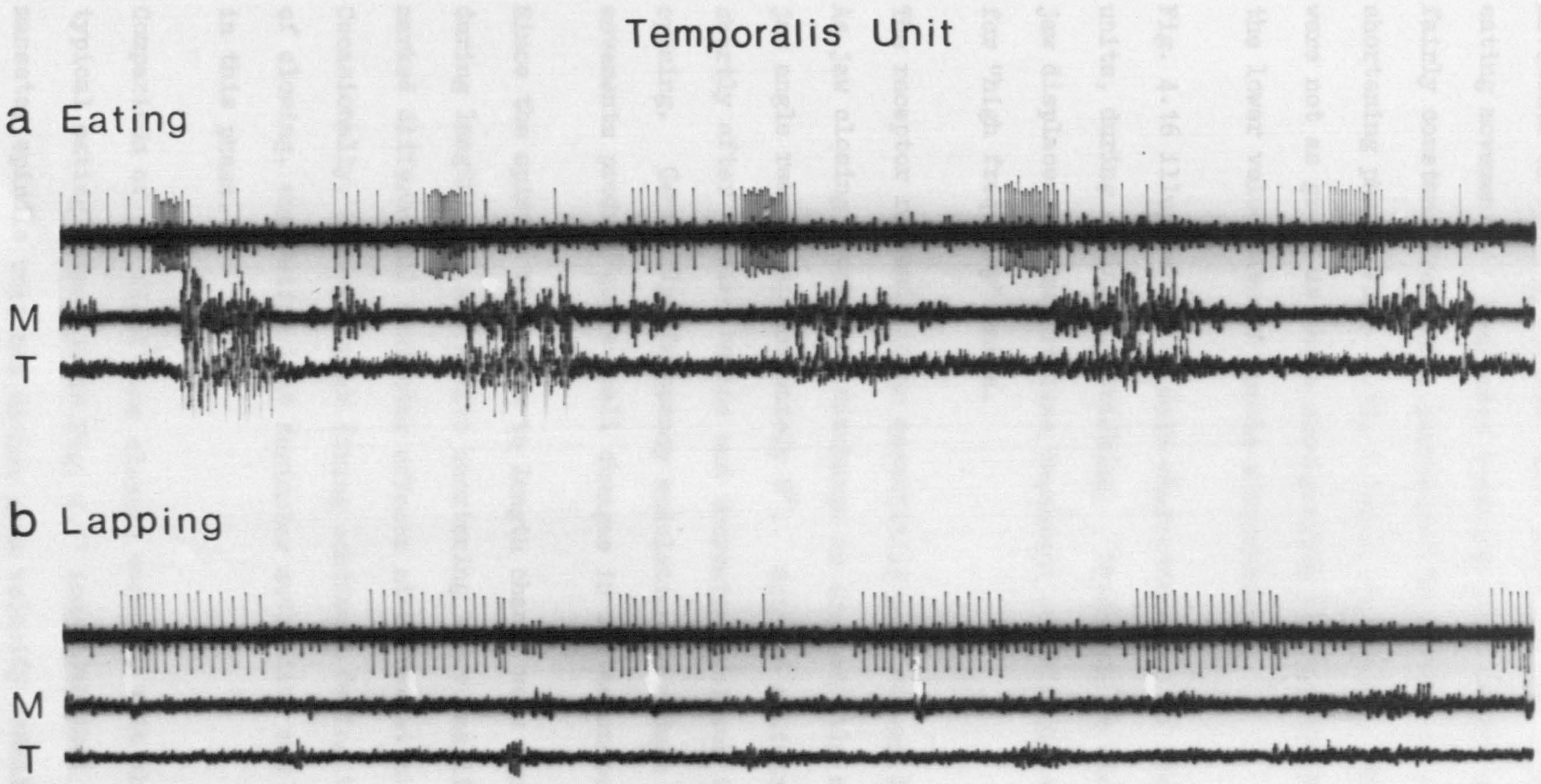


Fig. 4.14
A further record of the unit shown in fig. 4.13.

The discharge of high frequency spindle units during the small rapid movements of lapping contrast with those for the larger more variable eating movements. Responses consisted of a series of bursts of fairly constant frequency, punctuated by periods of silence during the shortening phase (Figs. 4.15, 1.13(b), 4.14(b)). Maximal frequencies were not as great as those accompanying eating, presumably because of the lower velocities of muscle stretching.

Fig. 4.16 illustrates the main characteristics of "low frequency" units, during eating and drinking. Discharge is closely related to jaw displacement, but it less dependent on velocity than was the case for "high frequency" units.

The receptor responds in an essentially passive way during eating. As jaw closing progresses discharge is consistently silenced when the jaw angle reaches approximately 8^{o}. Spindle activity reappears shortly after opening begins and thereafter follows the angle of opening. Generally, frequency modulation is modest and large jaw movements produce quite small changes in instantaneous frequency.

Since the spindle responses to length change were basically similar during lengthening and active shortening there was little evidence of marked differential fusimotor effects at different times in the cycle. Occasionally, however, peak firing continued during the initial part of closing, suggesting that fusimotor activation may have increased in this phase.

Comparison of the final slow closing movement with the previous typical eating movements in Fig. 4.17 indicates that this "low frequency" masseter spindle unit has either some velocity sensitivity or that there

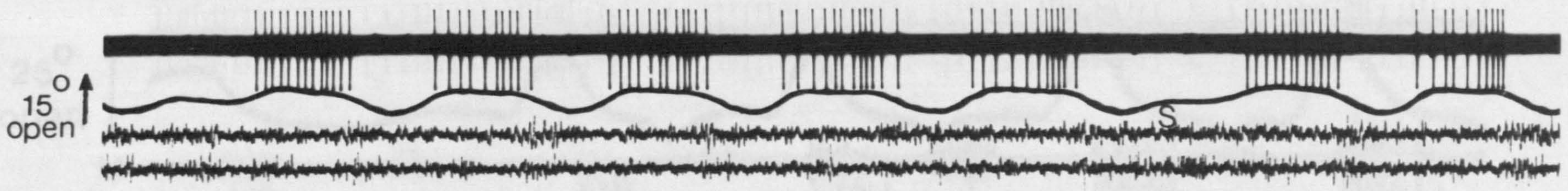


Fig. 4.15

Behaviour of a "high frequency" masseter unit during lapping, together with jaw displacement and EMG from masseter (M) and temporalis (T). Swallowing movements indicated by the letter S.

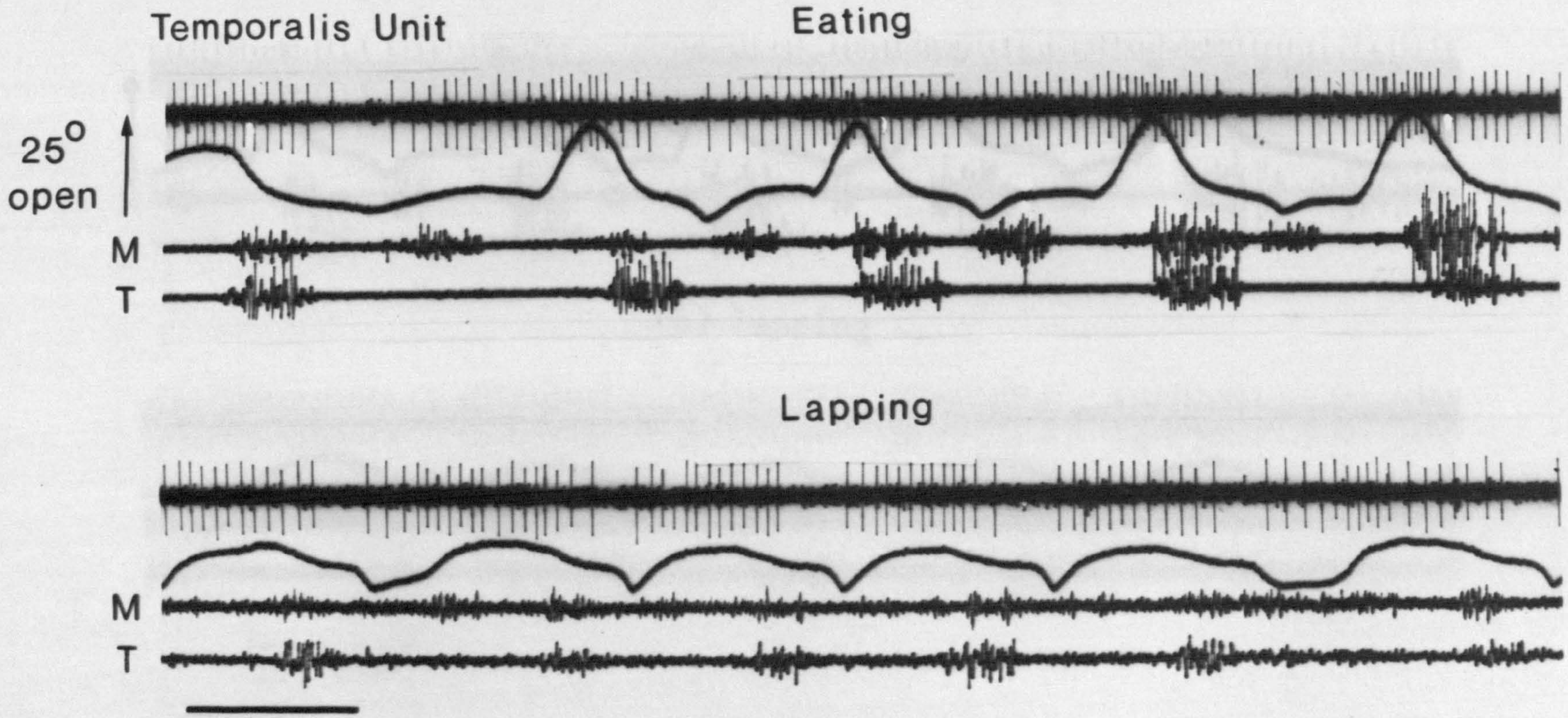


Fig. 4.16
Responses of a "low frequency" temoralis unit, together with jaw displacement and EMG from masseter (M) and temporalis (T), during eating and drinking.

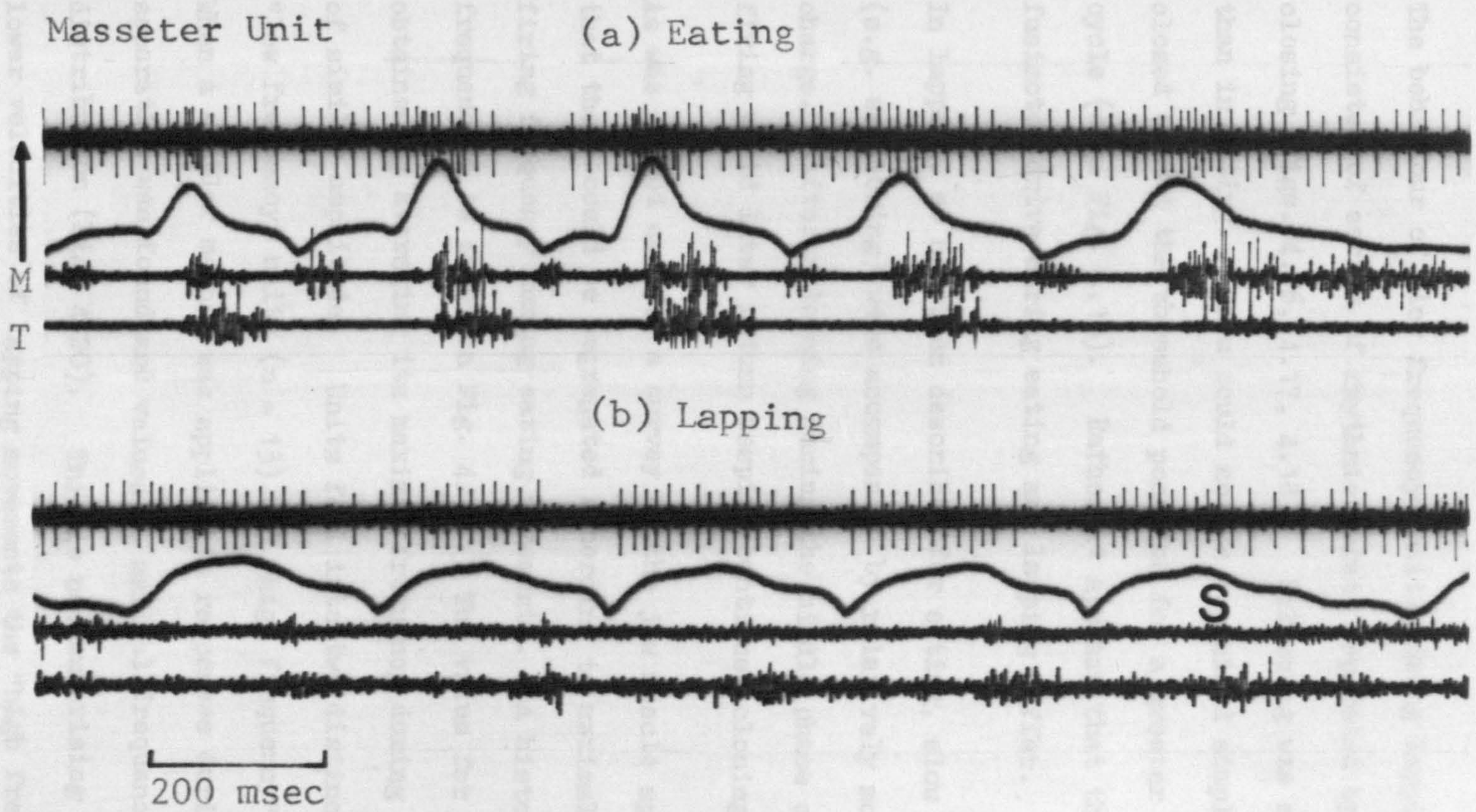


Fig. 4.17
Responses of a "low frequency" masseter unit during eating and drinking. The arrow represents 25° of jaw opening. EMG was recorded from masseter (M) and temporalis (T). Swallowing is shown by the letter S in record (b).

is increased fusimotor activity during the period of prolonged closing. At this time spindle discharge persisted although the jaw angle was one normally associated with the abolition of activity.

The behaviour of "low frequency" units during lapping movements consisted of series of rhythmic bursts separated by silencing during closing (Figs. 4.16, 4.17, 4.18). Silencing was more pronounced than in eating. This could not be explained simply by the jaw being closed beyond the threshold position for a greater proportion of the cycle (e.g. Fig. 4.18). Rather it appears that the patterns of fusimotor drive during eating and lapping differ.

In lapping, as has been described for eating, slow closing movements (e.g. swallowing) were accompanied by relatively more spindle discharge. After silencing during the middle phase of such a movement firing would later return despite continued closing (Figs. 4.17, 4.18).

As was stated earlier a survey of the jaw muscle spindle units indicated that these could be segregated according to maximal instantaneous firing frequency during eating movements. A histogram of maximal frequencies is shown in Fig. 4.19. The value for each unit was obtained by averaging its maximal frequency during ten movement cycles of similar amplitude. Units fell into two distinct groups, namely "low frequency" units (n = 13) and "high frequency" units (n = 8). When a similar method was applied to responses during lapping no such separation was found and values of maximal frequency formed a single distribution (Fig. 4.20). This is not surprising since during the lower velocities of lapping movements the "high frequency" units showed far less marked modulation.

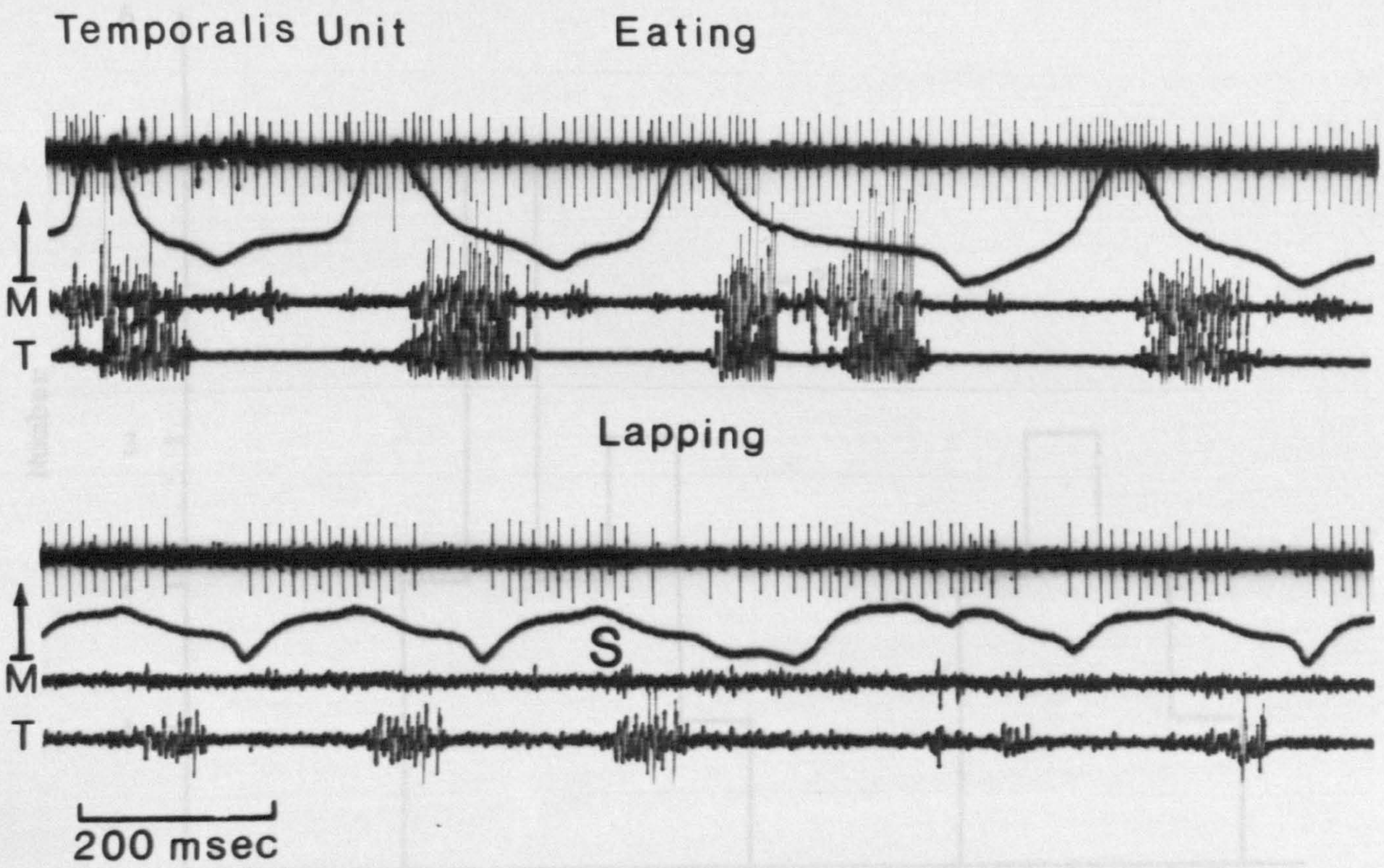


Fig. 4.18
Responses of a "low frequency" temporalis unit during eating and drinking. The arrow represents 25° of jaw opening. EMG was recorded from masseter (M) and temporalis (T). Swallowing is shown by the letter S in (b).

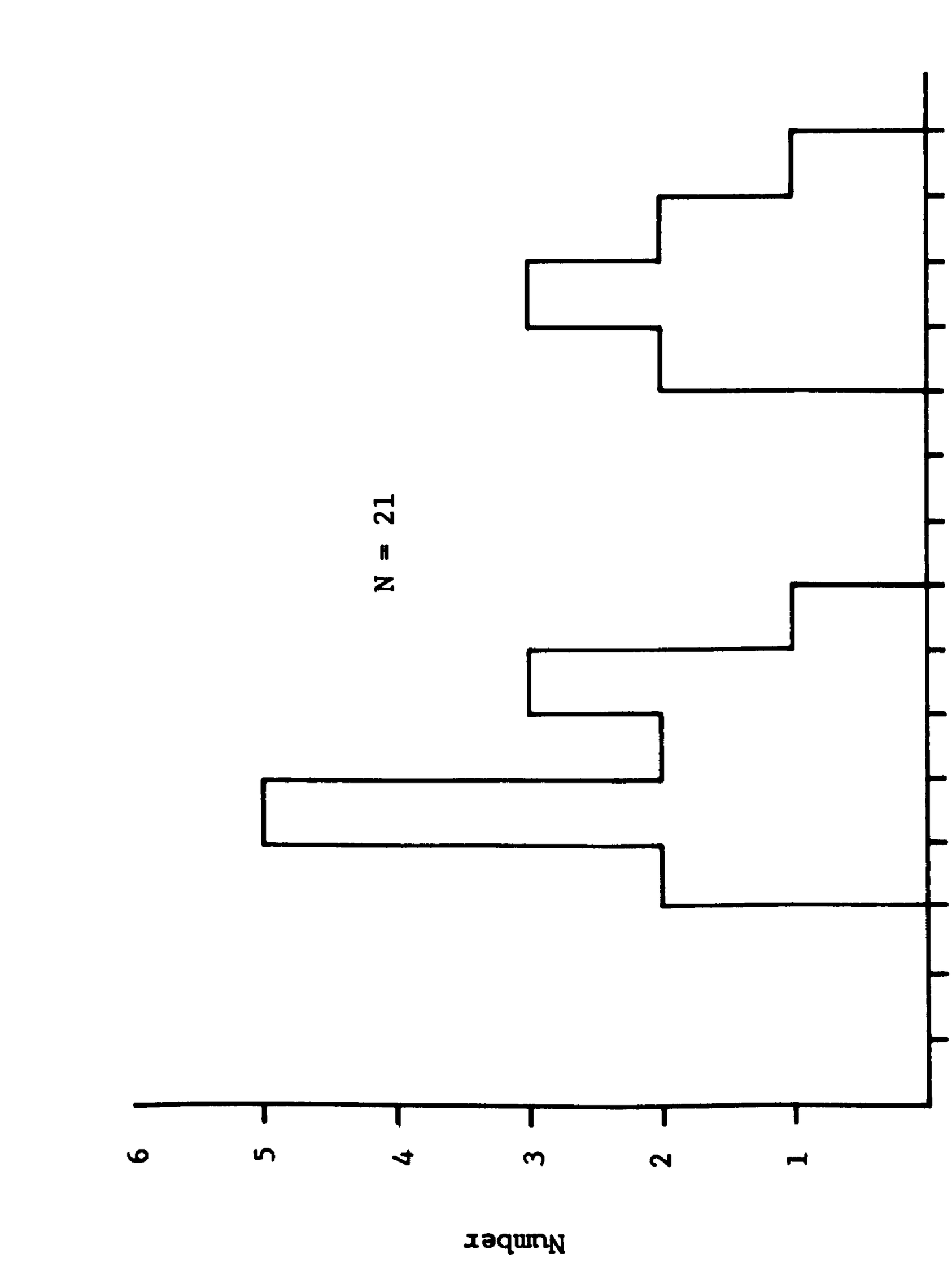


Fig. 4.19 Histogram of the maximal frequency of discharge of jaw muscle spindle units during comparable eating movements.

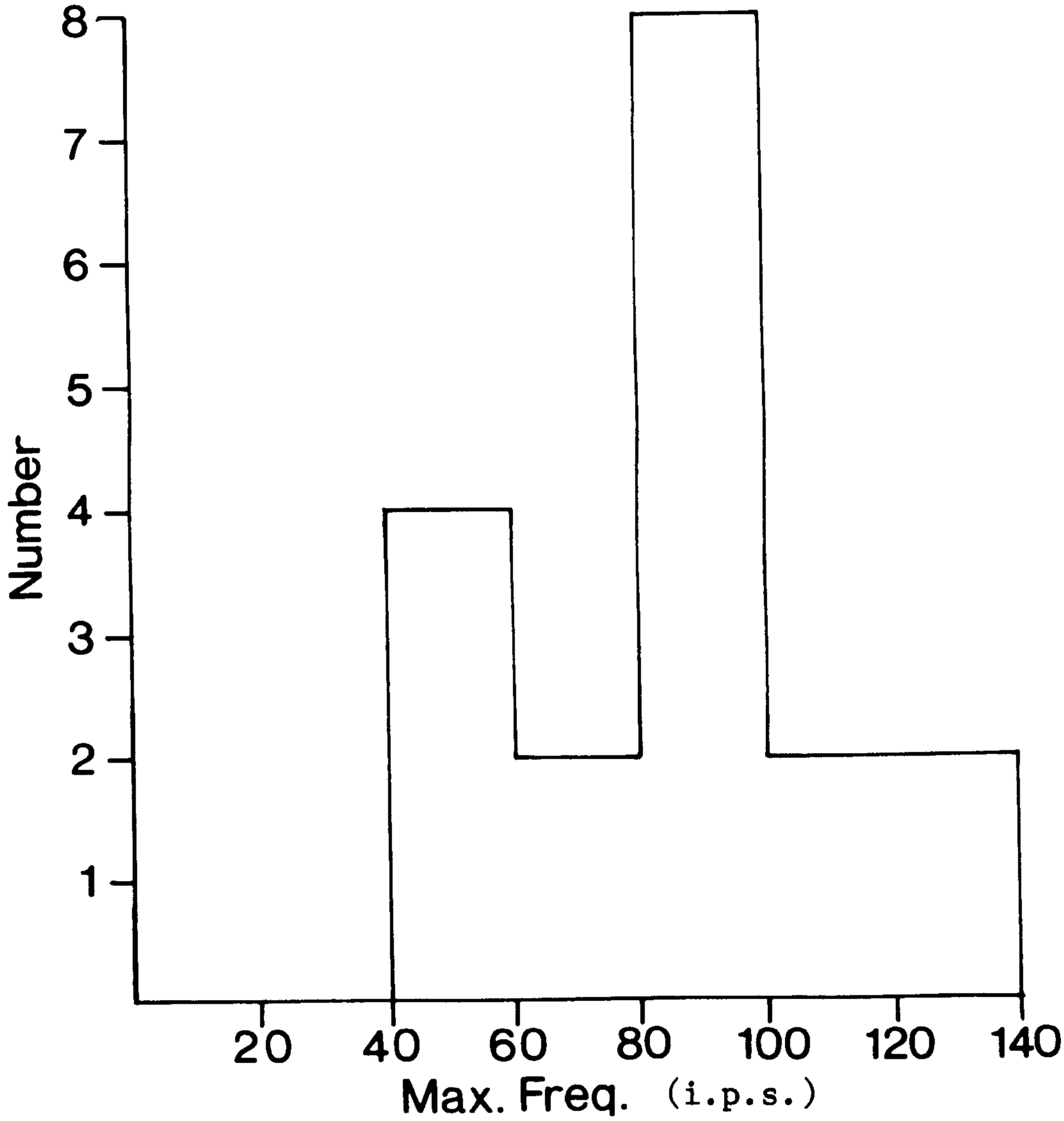


Fig. 4.20
Histogram of maximum firing frequencies of jaw muscle spindles during the repetitive movements of lapping. Units pooled from the three jaw-closing muscles.

4.3.4 Spindle Responses to Passive Manipulation of the Jaw

In a number of animals the effect of passive manipulation of the jaw was tried. Fig. 4.21 shows the responses of a "high frequency" temporalis unit to the application of muscle stretch interposed during regular jaw movements.

In each of the three trials (Fig. 4.21(a), (b) and (c)) passive opening was accompanied by a powerful sustained increase in spindle discharge. However, in no case did this result in an appreciable EMG response. During the subsequent active closing spindle firing was rapidly reduced.

The form of the response elicited by passive extension of the muscle was normally that illustrated, although the magnitude of the increase in spindle firing was variable. This presumably depended on the nature of fusimotor excitation.

4.3.5 Relationship between Spindle Discharge and Smoothed Rectified EMG (Sre)

If spindle discharge makes an appreciable contribution to α-motorneurone excitation, as proposed in the servohypothesis, it should be related to EMG activity during active contraction.

In Fig. 4.22 the responses of a "low frequency" temporalis spindle unit are shown in relation to smoothed rectified EMG from masseter (SreM) and temporalis (SreT) during eating. In the first eating cycle some spindle activity persists during the early part of the closing phase, but rapidly declines as the movement proceeds. The movement is accompanied by relatively large amplitude EMG activity in both temporalis and masseter. During the slower closing movement of the third cycle

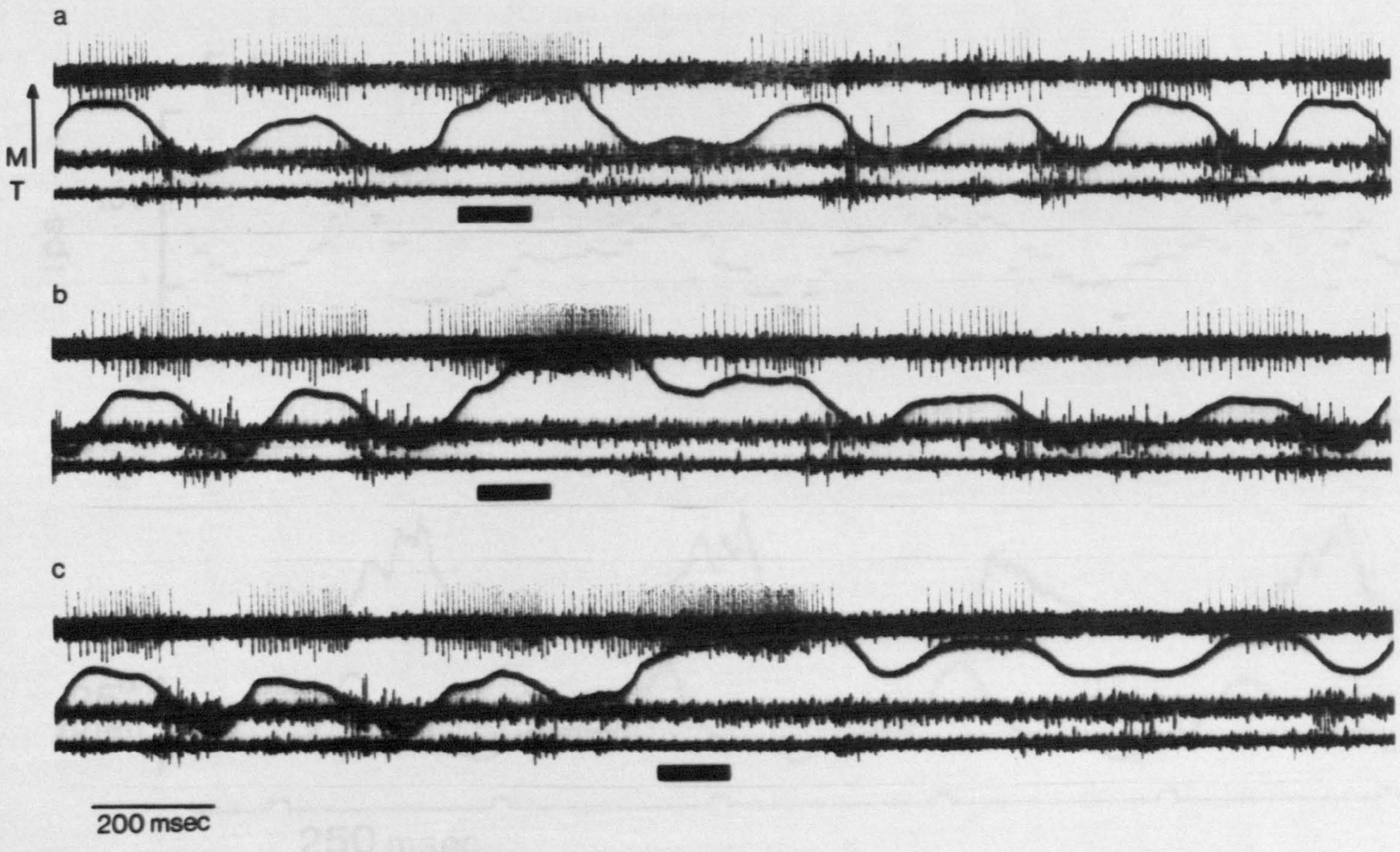


Fig. 4.21

The effect of passive opening of the jaw on the discharge of a "high frequency" temporalis unit. The arrow represents 25° of jaw opening. Masseter and temporalis EMG are labelled M and T. The thick bar indicates passive movements.

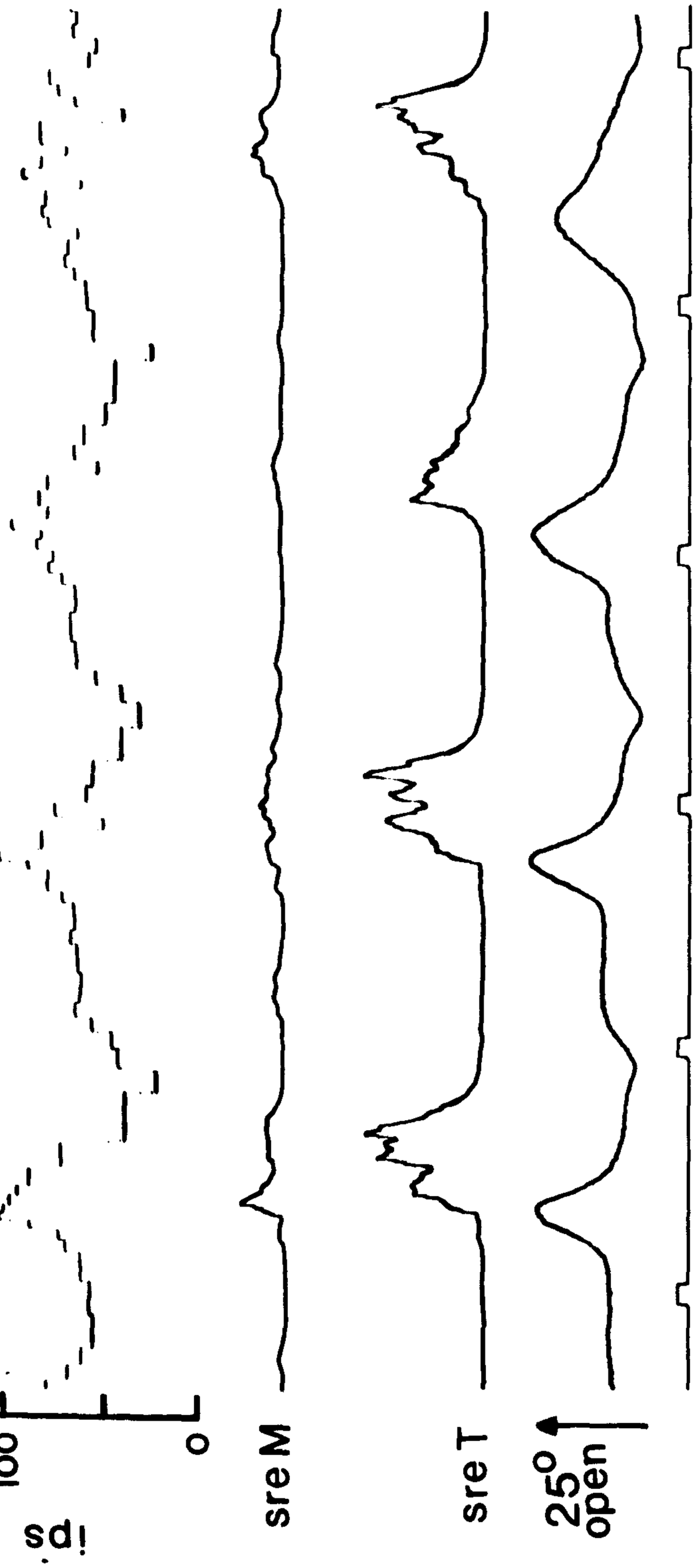


Fig. 4.22
Behaviour of a "low frequency" temporalis unit in relation to smoothed rectified EMG from masseter (sreM) and temporalis (sreT) and jaw displacement during eating. Spindle discharge is shown as an instantaneous frequency plot with sample and hold. EMG was smoothed with a time constant of 10 msec.

more spindle firing is present but EMG in both jaw-closing muscles is reduced.

Further examples of the variability of the relationship between "low frequency" spindle unitary discharge and Sre are found in Figs. 4.23, 4.24, 4.25(a). The "low frequency" temporalis unit illustrated in Fig. 4.23 gave a strong burst of firing at the beginning of the third rapid closing movement and yet SreT is small at that time.

Often rapid movements were accompanied by relatively little EMG activity in the jaw-closing muscles and vice-versa. This was interpreted as resulting from differences in the consistency of the food being eaten. In Fig. 4.25(a) the second eating cycle the shortening phase is faster than that of the subsequent movement despite reduced EMG. However, the temporalis unit activity is greater in the second closing movement suggesting that fusimotor excitation must have been stronger.

In the case of lapping (Figs. 4.25(a), 4.26) both movements and spindle discharge were more stereotyped. EMG activity was generally weak in both masseter and temporalis. Again considerable variability in the relationship between spindle firing and Sre was found. Comparing the first and second lapping cycles in Fig. 4.26 discharge is abolished more quickly in the closing phase of the former when there is a relatively large burst of EMG. In the second movement SreT is slightly reduced during closing although spindle firing is stronger in this period.

Generally "high frequency" spindle units were abruptly silenced during contraction of the jaw-closing muscles in masticatory movements. It is therefore unlikely that they could have provided significant α

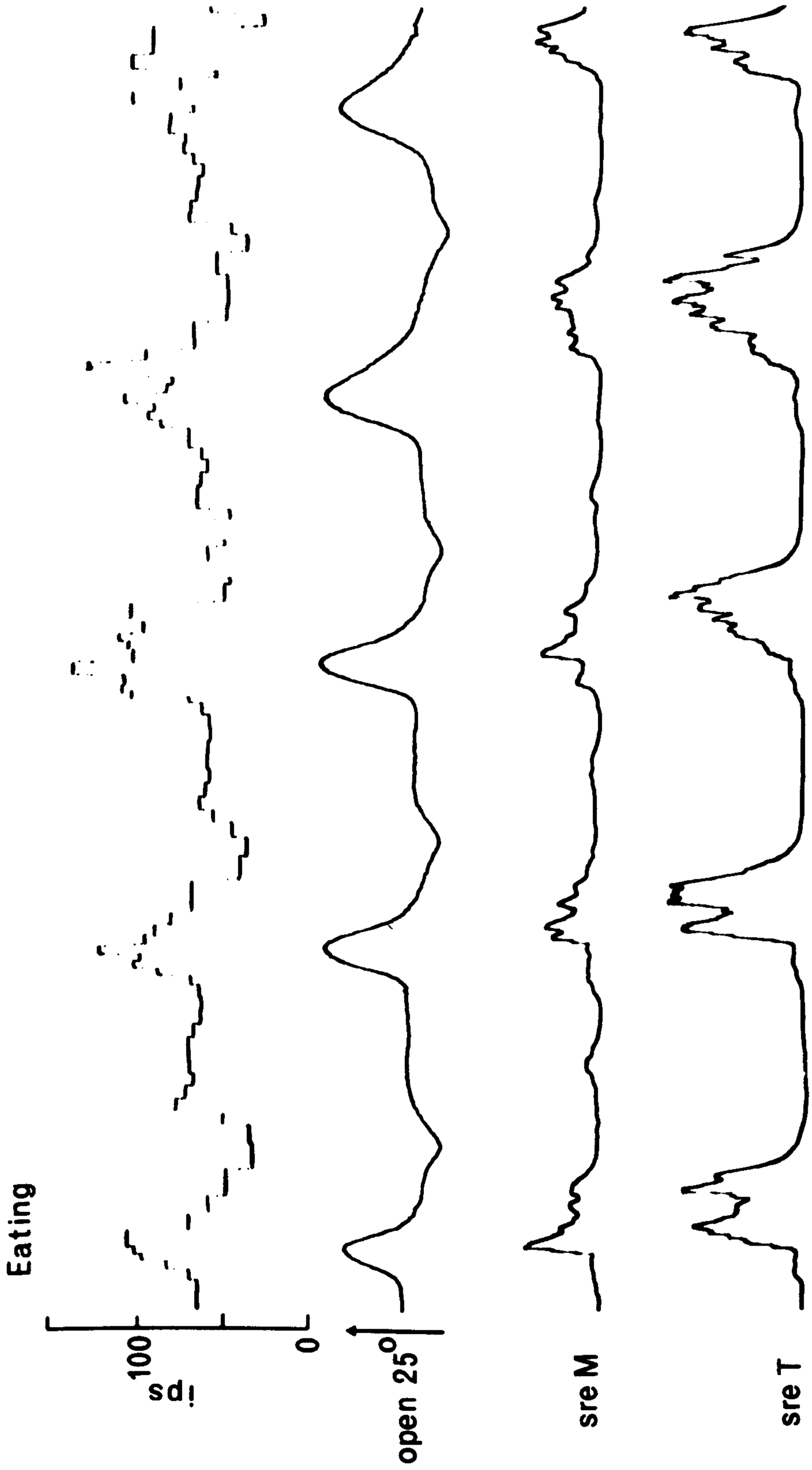


Fig. 4.23
Behaviour of a "low frequency" temporalis unit in relation to smoothed rectified EMG and jaw displacement during eating.

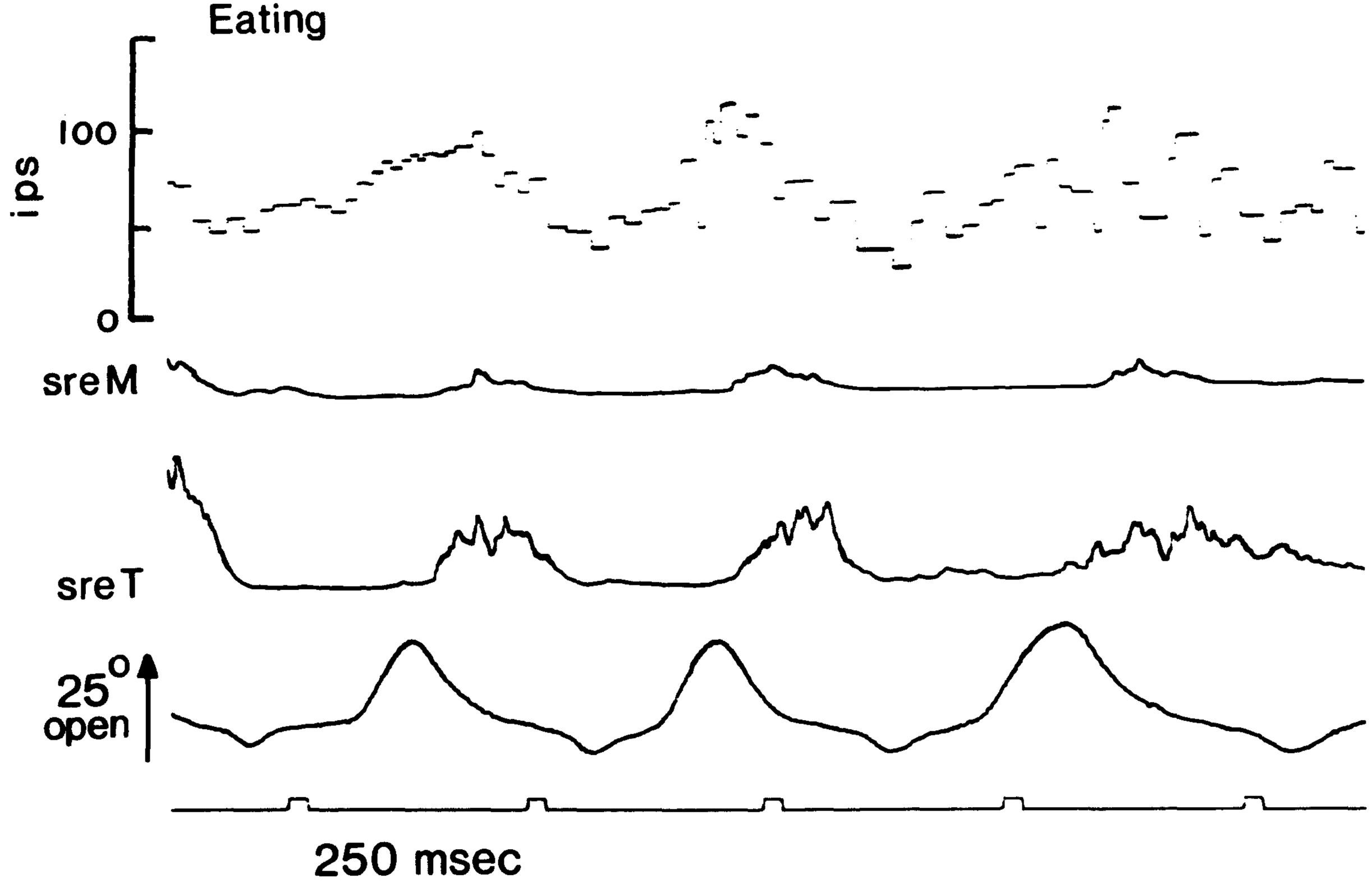


Fig. 4.24

Behaviour of a "low frequency" temporalis unit in relation to smoothed rectified EMG and jaw displacement during eating.

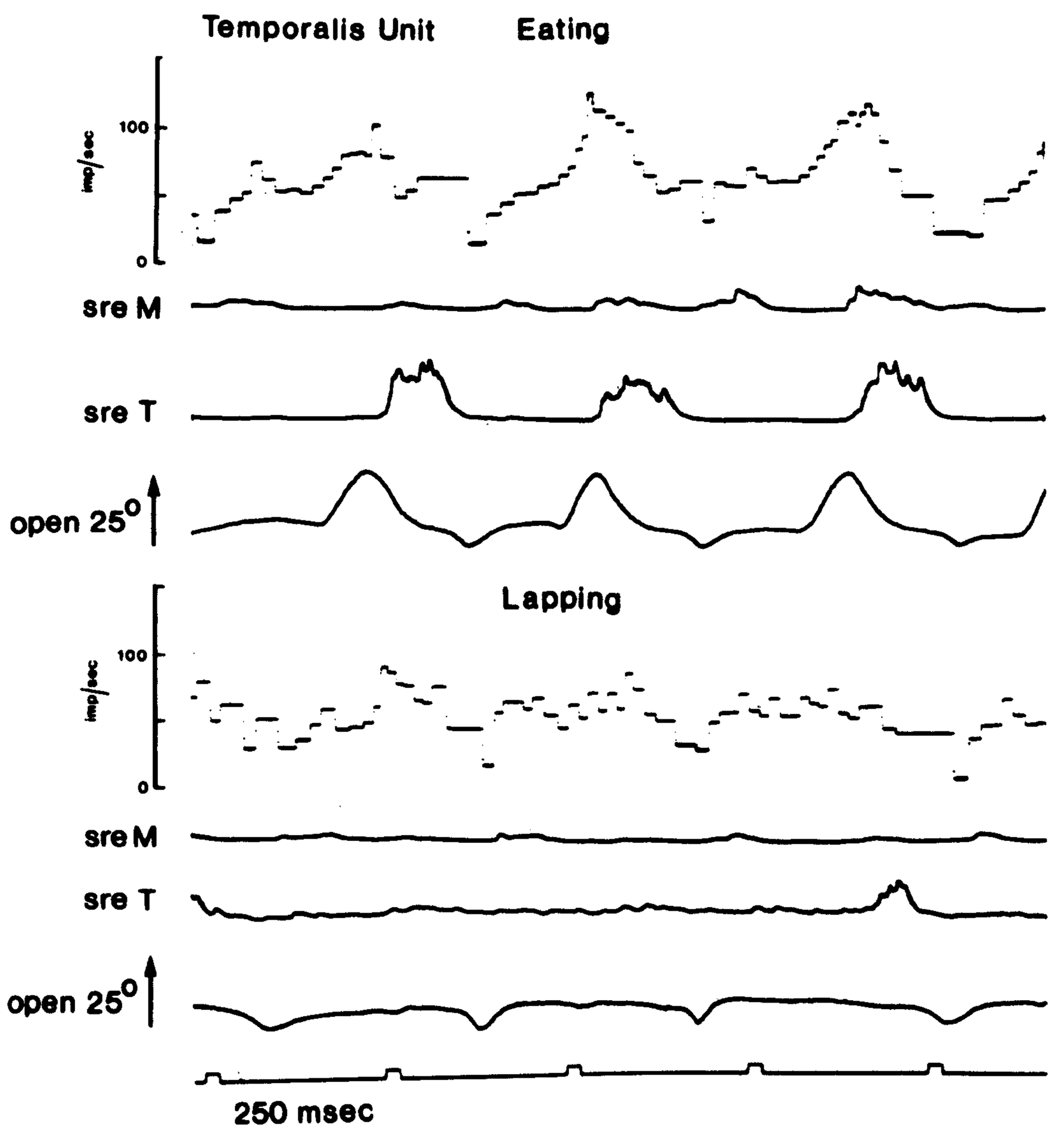


Fig. 4.25

Behaviour of a "low frequency" temporalis unit in relation to smoothed rectified EMG and jaw displacement during (a) eating and (b) drinking.

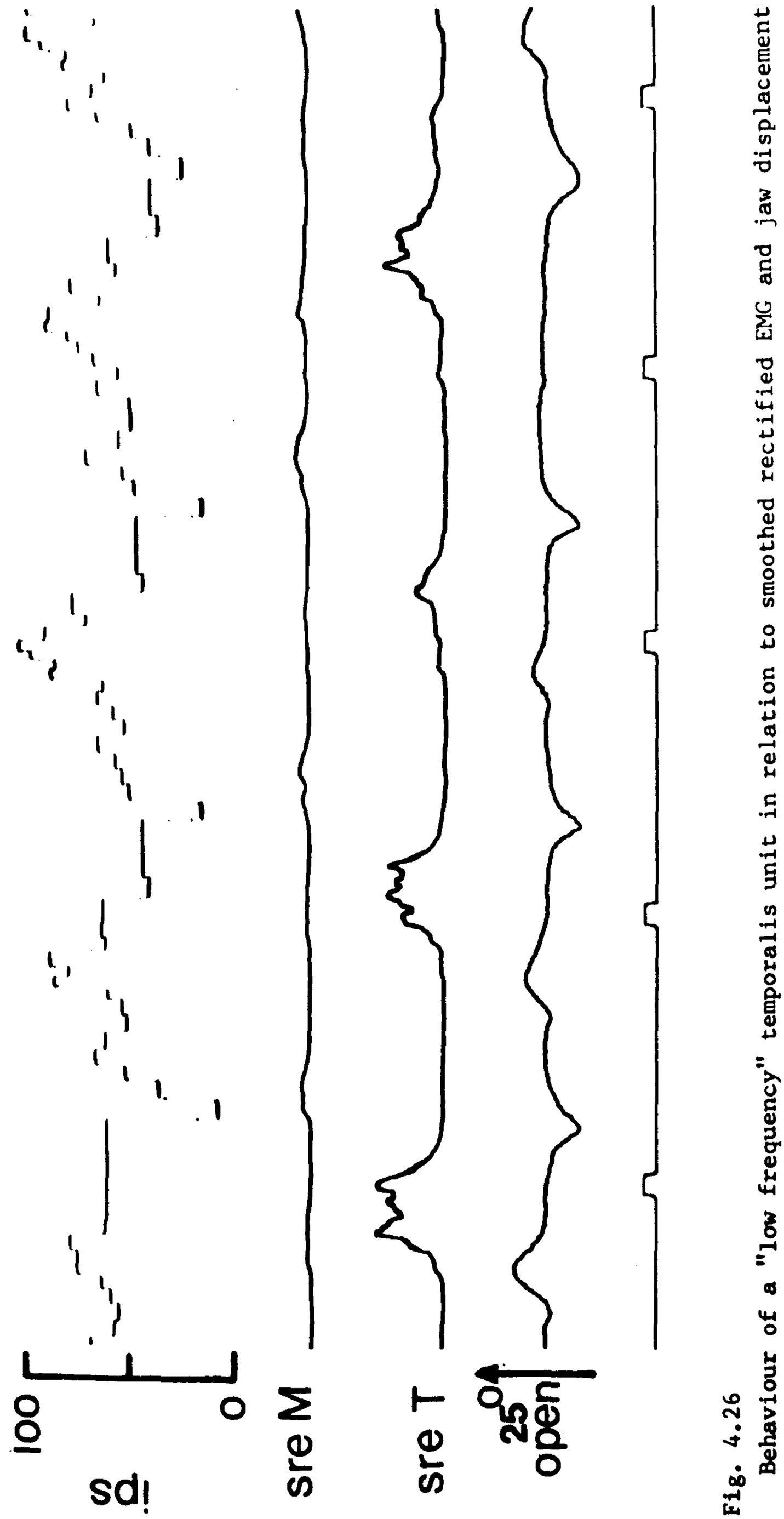


Fig. 4.26
Behaviour of a "low frequency" temporalis unit in relation to smoothed rectified EMG and jaw displacement during drinking.

motorneurone excitation for the majority of the contraction. The extremely dynamically sensitive temporalis unit shown in Fig. 4.27 demonstrates this behaviour. A second temporalis unit (Fig. 4.28) does show some firing at the beginning of the EMG activity, but is silent for most of the contraction. Also the relationship between spindle activity and Sre is variable in successive movements, as was the case for "low frequency units".

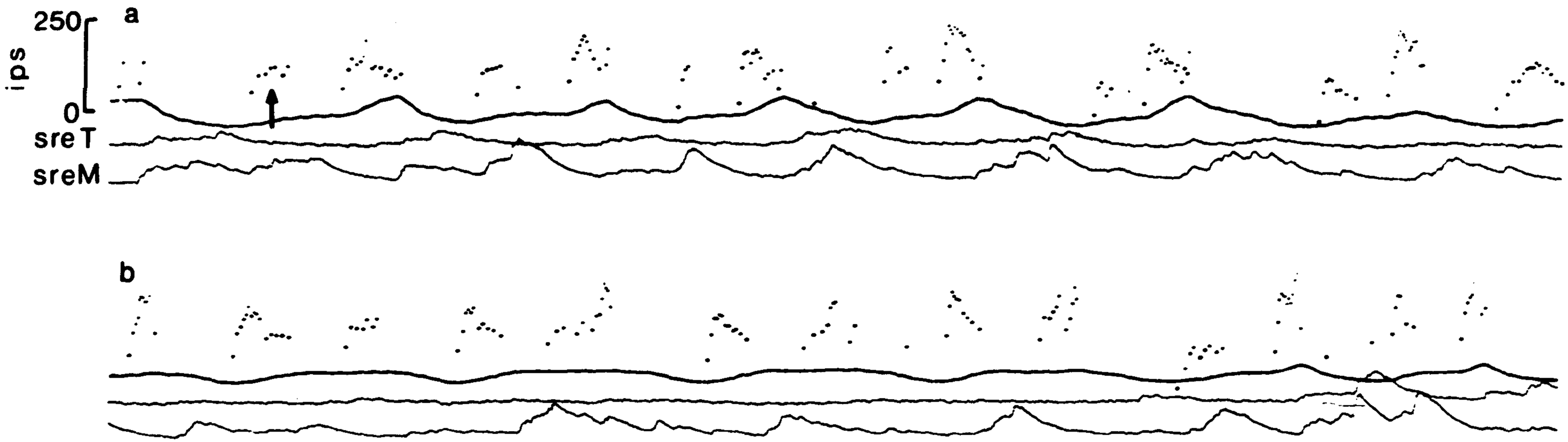


Fig. 4.27

Behaviour of a "high frequency" temporalis unit in relation to smoothed rectified EMG and jaw displacement during eating. The vertical arrow represents 25° of jaw opening. Spindle discharge is shown as instantaneous frequency.

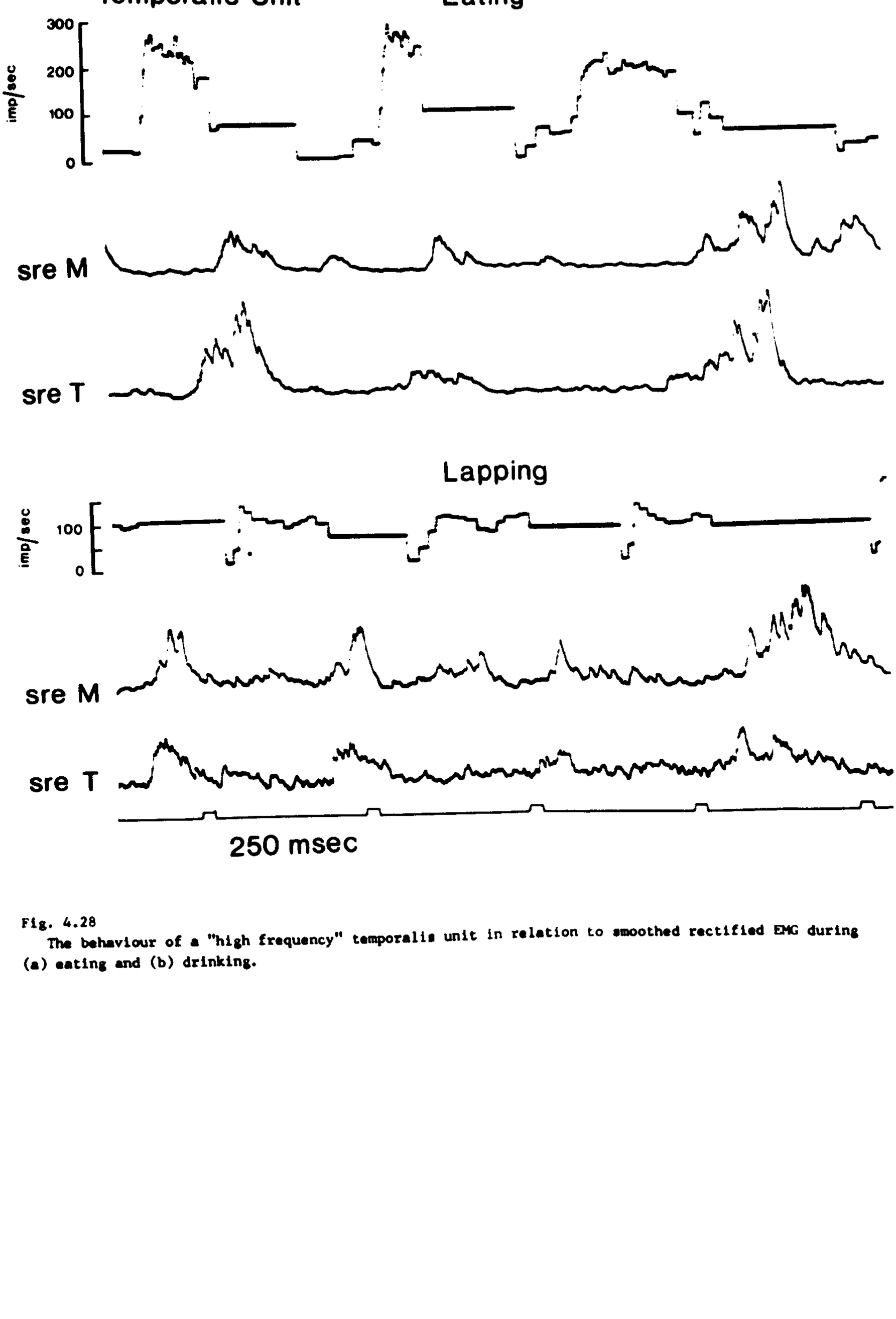


Fig. 4.28
The behaviour of a "high frequency" temporalis unit in relation to smoothed rectified EMG during (a) eating and (b) drinking.

4.4 DISCUSSION

Three main points emerged from the present studies on jaw-closing muscle spindle behaviour in the conscious cat during the normal movements of eating and drinking. Firstly, the spindle responses commonly resembled those of the passive receptors in their general form, i.e. increased discharge during extension and reduced discharge during active shortening. Nevertheless, there was clear evidence of fusimotor action during contraction. Secondly, spindle units could be divided into two groups according to their peak firing frequency during comparable movements. The pattern of responses of these two groups suggest that they correspond to primary and secondary endings. Thirdly, there was little evidence of spindle participation in reflex compensation during the patterned masticatory movements studied.

The presence of fusimotor excitation of spindles during contraction was occasionally dramatically demonstrated by the virtual absence of frequency modulation during large movements, indicating close $\alpha - \gamma$ matching. This type of behaviour was best seen in relatively slow movements, e.g. licking the lips. In the rapid stereotyped movements of eating and drinking, fusimotor effects were far less obvious and rhythmic spindle fluctuations occurred, often with complete silencing. During these movements the discharge of static "low frequency" units closely followed muscle length whereas that of "high frequency" units was related principally to velocity. The tendency for the firing of many "low frequency" units to remain relatively high during the early part of closing suggests that increased fusimotor drive may have been present during this period, although this is difficult to assess in the absence of direct muscle length recording. Comparison of different categories of movement suggested that these were associated with different patterns of fusimotor activity.

The present results contrast markedly with those obtained during twitch and sustained isometric contractions of wrist and finger flexor muscles in awake human subjects. Under these conditions spindle discharge generally increased during contraction, although little activity had previously been present in the relaxed state (Hagbarth & Vallbo, 1968; 1969; Vallbo, 1971). Occasional variations from this pattern of behaviour were seen and several units showed conspicuous silencing during the phase of rising tension and isometric twitches (Vallbo, 1971). This was most common at low contraction strengths and was often reversed in stronger contractions. The overall conclusion from these studies was that some form of "α - γ co-activation" was present during voluntary isometric contractions.

However, in more recent experiments in which spindle responses were recorded during small, slow movements of the "ring finger", active shortening was accompanied by a reduction in spindle firing (Vallbo, 1973, Fig.5). The ending, believed to be a primary, was silenced for approximately 0.25 sec during the early part of contraction. This silencing occurred despite a prior increase in spindle firing, accompanying the appearance of EMG activity, before the commencement of the shortening movement. Subsequently, during the period of sustained shortening spindle discharge progressively increased. These observations suggest that in the isotonic situation too some form of α - γ co-activation was present. Towards the end of the record as EMG activity declined spindle firing again silenced and, surprisingly, remained low even during muscle lengthening.* In an even slower

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* In this record (Vallbo, 1973, Fig.5) movement lags over 1 sec behind the appearance of EMG activity. It seems possible therefore that either movement was not faithfully recorded or that EMG recording was not restricted to the muscle(s) responsible for the movement.

shortening movement (Vallbo, 1973, Fig. 6) discharge of the same spindle ending increased during contraction but did not closely follow joint angle. Instead, firing frequency appreared to be inversely related to the velocity of shortening.

Matsunami & Kubota (1972), recording from the region of the MeNV in the restrained monkey during masticatory movements, obtained responses that they attribute to jaw muscle spindle units. Unfortunately, several factors hamper the interpretation of their records. The relation between unitary discharge and jaw muscle (masseter) contraction was extremely variable. About half the units showed decreased discharge during contraction, whilst the activity of the remainder increased at this time.

However, in the absence of a jaw displacement record (Matsunami & Kubota, 1972, Fig. 5), especially in view of the complexity of jaw movements in the monkey, it is impossible to tell how muscle length was changing. Furthermore, in no case was the muscle of origin of presumed spindle afferents determined and yet only masseter EMG was recorded.

The reduction in spindle firing during active shortening observed in the present experiments and the human in flexor digitorum (Vallbo, 1973) contrasts with previous findings in anaesthetized and decerebrate animals. Under these conditions, during certain reflex motor acts, fusimotor excitation can be sufficiently powerful to produce speeding of spindles during contraction (Granit & Kaada, 1952; Eldred, Granit & Merton, 1953; Eldred & Hagbarth, 1954). Spindle frequency has also been shown to increase during spontaneous contraction of the respiratory muscles of the cat (see Sears, 1973).

Severin, Orlovskii & Shik (1967) recorded the responses of spindles of the hindlimb muscles of the decerebrate cat during locomotion produced by caudal midbrain stimulation. Spindles in the ankle extensors discharged at the highest frequencies during the contractions of these muscles associated with the stance phase of the step cycle. The initial part of this phase involves a lengthening contraction of the ankle extensors, so that extension of these muscles could be responsible, at least in part, for the increase in spindle discharge. Subsequently, however, spindle activity remained high despite shortening, suggesting that fusimotor activation increased.

The other interesting observation was that the spindles were not excited appreciably by the large passive lengthening movements that occur during the swing phase. Goslow, Stauffer, Nemeth & Stuart (1973), recording the responses of de-efferented spindles of these muscles, have shown that the low passive responsiveness of the receptors found by the Russian group is not attributable to an inadequate extent, range or rate of stretch. It is therefore difficult to explain the low receptor sensitivity seen in the experiments of Severin, Orlovskii & Shik (1967) simply on the basis of reduced fusimotor drive, as was proposed.

Alternative possibilities, in view of the similarity in the patterns of response of presumed spindles and tendon organs (Severin, Orlovskii & Shik, 1967; Figs. 2 and 3), are that either the receptor types or their muscles of origin were not correctly identified.

In this context the observations most directly related to the present work are those of Taylor & Davey (1968). Jaw closing muscle spindle activity was recorded in cats recovering from anaesthesia during movements initiated by the introduction of fluid into the mouth. Later, after the administration of additional anaesthetic to suppress fusimotor activity, the responses of the same unit were recorded during identical passive movements of the jaw.

In the active movement, although spindle discharge occurred principally during the lengthening phase, some firing was also present during shortening (Taylor & Davey, 1968, Fig.2). This contrasted with the passive situation when the spindle was totally silenced during shortening and firing only reappeared during extension. The difference between these two patterns of spindle behaviour was interpreted as a measure of the fusimotor effects and it was concluded that an increase in fusimotor drive must have occurred during contraction.

A possible explanation for the discrepancies between the results obtained in conscious animals and those in anaesthetized or decerebrate animals lies in the inevitable disturbances of the motor system found in the latter states. Both anaesthesia (Koeze, 1968; Vedel & Mouillac-Boudevin, 1970) and decerebration, especially by mid-collicular section (Matthews, 1958), tend to favour fusimotor over α motor effects.

Certainly, in the records of Taylor & Davey (1968), in very lightly anaesthetized cats, fusimotor discharge, as judged by the irregularity of spindle firing at constant length, must have been very variable. High frequency spindle bursts, almost as great as those associated with active movements, occurred in the absence of appreciable muscle length changes.

In view of the disorganized nature of the motor system during anaesthesia or decerebration the relevance of results obtained under these conditions to the intact animals is doubtful.

The present experiments in the conscious cat show evidence of two groups of spindle afferent units. "High frequency" units, because of their high dynamic sensitivity seem likely to correspond to primary endings. Conversely,the responses of "low frequency" units were for more static suggesting that these were secondaries.

In the human studies (Hagbarth & Vallbo, 1968, 1969; Vallbo, 1971, 1973) it is thought that exclusively primary afferents have been sampled. This belief is based on the variability of firing of the receptors and on their dynamic responsiveness to passive extension. These receptors rarely attained frequencies in excess of 100 i.p.s. in contrast to those of 220-320 i.p.s. for "high frequency" cat jaw muscle spindle units. These differences may reflect differences in the amplitudes and velocities of movement in the two situations.

The behaviour of presumed primary spindle endings of the cat jaw muscles and of human finger muscles during active movement suggest a predominance of γ_d activity during contraction. This would account for the abrupt silencing of spindles during shortening, since γ_d stimulation enhances the velocity sensitivity of primary endings, as well as their static firing, in anaesthetized cats (Crowe & Matthews, 1964; Brown, Crowe & Matthews, 1965). If appreciable γ_s driving had also been present greater persistance of spindle discharge would be expected since these fusimotor fibres reduce dynamic responsiveness (Brown & Matthews, 1966) and are known to be capable of maintaining spindle discharge in the face of muscle shortening (Lennerstrand & Thoden, 1968). An increase in γ_d excitation shortly after the appearance of EMG activity, but before the commencement of shortening, could account for the increase in static firing seen in the human study (Vallbo, 1973).

In the case of presumed secondary jaw muscle spindle units, evidence from cat hindlimb muscles suggests that these may be under the influence of almost exclusively γ_s innervation (Appelberg, Bessou & Laporte, 1966). The tendency for spindle discharge to continue, albeit at a reduced frequency, during an initial phase of shortening is consistent with the presence of γ_s activation.

Whilst fusimotor activity was obviously present during voluntary jaw movements, there was no indication of it having been responsible for initiating contraction as proposed in the "follow-up length servo" hypothesis (Merton, 1951). The production of extrafusal contraction in this way demands that increased spindle activity precede the onset of α-motor discharge. Thereafter, if contractions were being driven via the γ-route, α-motor firing should closely follow spindle discharge. In the present experiments these criteria were not fulfilled and the hypothesis may be rejected, at least in this form. The observations were only consistent with the initiation of contraction by the direct action of descending inputs on the α-motorneurones.

Similar conclusions were reached by Vallbo (1971) for human isometric contractions and for jaw movements in the conscious monkey (Matsunami & Kubota, 1972). In neither case did spindle firing consistently precede EMG activity.

These recent findings greatly reduce the interest of previous reports of γ leading in anaesthetized animals (Hunt, 1954; Granit & Kaada, 1952; Eldred & Hagbarth, 1954).

Neither did results from the cat jaw muscle spindles provide support for Phillip's (1969) theory that balanced α and γ activation maintains constant spindle firing provided movements go according to plan. According to this scheme any deviations from the "expected" course cause changes in spindle discharge which produce compensatory modifications of contraction. Although a few examples of such α - γ matching during large movements were seen, e.g. licking the lips, these were exceptional. Certainly during the stereotyped movements of eating and lapping rhythmic spindle fluctuations were regularly found. The latter

movements, in view of their repeatable nature and well known load, might be considered a particularly suitable situation in which to look for α - ϒ matching. Similarly in Vallbo's (1971, 1972) work on human muscle spindles there was little evidence of α - ϒ balancing.

Obviously neither the evidence against the "follow-up length" servo nor α - ϒ matching invalidates the operation of a muscle length servo mechanism. Taylor (1972) argues, from systems analysis considerations, that the advantages of servo action are retained if the command signal is largely via the α-motorneurones, provided there is sufficient fusimotor excitation to prevent protracted silencing of spindles and thereby development of an "open loop" situation.

Even this is not strictly essential since during contraction negative feedback is also provided by muscle receptors in the antagonists.

Nevertheless, the "α - ϒ linkage" concept (Granit, 1970) has been widely seen as a solution to the problem of spindle silencing during shortening. By analogy with the amphibia in which there is built-in α - ϒ linkage, as a consequence of the intrafusal fibres being supplied by branches of the main motor nerves (Katz, 1949; Gray, 1957), some form of coactivation is to be expected in mammals.

However, direct recording from human finger muscle (Vallbo, 1973) and cat jaw muscle spindles show that during shortening there is little or no discharge. Also whilst fusimotor activity accompanies contraction its relationship to α-motor activity is complex. Vallbo (1973) found that fusimotor activity, as judged by spindle discharge during isometric contractions, followed neither EMG activity nor force. Commonly at the start of contraction fusimotor excitation was relatively more powerful than α-motor discharge, whereas later when EMG activity was maximal spindle firing decreased. These findings suggest that the concept of a rigid α - ϒ linkage is too simple.

The present results agree with those of Vallbo (1973) in that fusimotor activation, certainly of presumed secondary endings, seemed to be greatest at the beginning of contraction. Also there was a very variable relationship between spindle activity and EMG.

The evolution of a separate fusimotor system by mammals, with dynamic and static subdivisions (see Matthews, 1972) is best justified as a means of producing a more flexible control system commensurate with the greater complexity of motor acts in higher animals. In principle the presence of length, velocity and force feedback, whose transducer characteristics can be independently adjusted, together with an on-line central computing capability would permit the system to be organized as an optimal controller (P. Landers, personal communication). Spindle gain could thus be regulated by fusimotor control to be appropriate to the mechanical properties of the load and to the precision or urgency of the motor task.

Despite the appeal of such theoretical considerations several lines of experimental evidence cast doubt firstly on the mode of operation of such a system and secondly on its effectiveness.

The present experiments showed little indication that cat jaw muscle spindles made any significant contribution to load compensation during eating and drinking. No simple relationship could be seen between the firing of individual spindles and Sre. On some occasions small amplitude EMG activity produced large rapid movements, probably due to the particular piece of food being of unexpectedly soft consistency. Yet such contractions were often accompanied by a relatively large burst of spindle firing. In contrast, at other times, presumably when the food was tougher, powerful muscle excitation resulted in only small movements and spindle discharge was surprisingly reduced. These findings seem to

contradict the prediction from a servo model, acting at the spinal level, that since the "error signal" driving muscle contraction is the sum of decending and spindle feedback excitation, changes in spindle frequency should be reflected in EMG activity.

As previously pointed out, in the human studies (Vallbo, 1973), during isometric contractions spindle afferent discharge was often greatest during little EMG activity and vice-versa. Also spindle fluctuations occurred whilst changes in contraction intensity were minimal.

It may, of course, be argued that the lack of a simple relationship between individual spindle discharge and EMG is not good evidence against spindle participation in servo control. The excitability of α-motorneurones is likely to be constantly varying in a complex way thereby altering the effectiveness of the input of muscle receptors in producing firing. In this way it is possible to see how presumed secondary jaw muscle spindle units could contribute to excitation, since they continue to fire during the early part of contraction, at a time when α-motorneurone excitability would be expected to be high. This assumes that secondary afferents have excitatory effects on synergistic motorneurones. Recent evidence in the decerebrate cat indicates that secondary endings of the soleus make a positive contribution to the tonic stretch reflex (McGrath & Matthews, 1973) although in view of earlier controversy (see Grillner, 1970, 1972) further clarification would be desirable.

Excitation, provided by "low frequency" units during the early part of contraction seems intuitively appropriate in providing the rapid activation of motor units necessary to overcome internal resistances of the muscle and the inertia of the load at the beginning of shortening.

However, it is difficult to see how presumed primaries could be acting in this way in view of their abrupt silencing during contraction.

The potential importance of the spindle feedback from antagonists must not be ignored in this situation. In the present study the majority of spindle discharge of both "low" and "high" frequency units occurred during the stretching phase. The negative spindle feedback to a particular α-motorneurone pool must be the sum of reduction in excitatory input from synergists and the increase in inhibitory input from the antagonists. It must be remembered, however, that in the human spindle studies during isotonic contractions (Vallbo, 1973, Fig. 5) discharge was reduced during lengthening as opposed to the increase seen in the present work.

To gain a fuller understanding of the role of muscle spindles in controlling contraction a better knowledge of the behaviour of the whole population during disturbances to movement is essential, together with further information of the extent of their reflex excitatory capabilities.

In experimental animals, maximal stimulation of muscle spindles evokes only a modest reflex contraction (Matthews, 1966), whilst Clough, Kernell & Phillips (1968) have shown that, in the baboon, the projection of spindle afferents is not confined to functionally related motorneurones. The latter observation suggests that spindle mediated spinal responses are likely to be relatively non-specific.

Several experiments designed to measure the efficacy of servo control of various human muscles during disturbances of contractions emphasize that the main response is not of spinal origin, that the gain of the system is rather low, that the compensation depends on the presence of cutaneous and joint sensation.

Early experiments on the extension (Hammond, Merton & Sutton, 1956) or release (Angel, Eppeler & Iannone, 1965) of a steady contracting muscle produced the anticipated response, i.e. increased contraction and silencing of EMG respectively. However, in both cases the latency, for

the main responses of these forelimb muscles was 40-50 msec, a value longer than that expected for a purely spinal mechanism but far less than that of a voluntary reaction. Marsden, Merton & Morton (1971) extended these observations by measuring the compensatory response to loading, unloading or halting a flexion movement of the thumb.

In each case a change in EMG, in the appropriate direction, occurred with a latency of approximately 50 msec. Surprisingly in the case of increased resistance the integral of the averged Sre simply shifted to a new gradient, indicating an increased steady level of EMG activity. During unloading complete silencing of EMG occurred for some 50 msec. These responses were interpreted as being due to muscle spindles kept operative by appropriate fusimotor driving (see Marsden, 1973).

However the effect of desensitizing the thumb, by local anaesthetic or occlusion of the circulation, reduced or abolished the compensatory responses. This occurred despite the muscle itself (flexor pollicus longus) being situated in the forearm. The conclusion reached was that both cutaneous and joint sensation were important for the normal functioning of spindles.

Stephens (personal communication) has recently done some preliminary experiments on the effect of sudden graded loading or unloading of the index finger during maintained isotonic contractions of the 1st dorsal interosseous muscle. As in previous studies an involuntary response was seen after some 40 msec. The interesting observation was that the amplitude of the EMG response did not appear to depend on the size of the change in external loading over a wide range. In the case of unloading the EMG was completely turned off for some 40-50 msec, but reappeared before the finger had returned to its original position. For loading an increase in contraction occurred for about 80-100 msec, by which time

the finger position was restored. These non-specific stereotyped reactions are quite different from those anticipated from a good positional servo in which the amplitude of the response would be expected to depend upon that of the disturbance.

In view of the evidence against pronounced spindle reflex action at the α-motorneurone level during normal voluntary movements, the intriguing question remains as to the use made of spindle input by higher motor centres, e.g. cerebral cortex and cerebellum. An obvious possibility is in the generation or up-dating of motor programs. The cerebellum, because of its proposed computational capabilities (Eccles, 1973) seems especially well suited to such a role.

The present evidence from the cat jaw muscles that spindles are indeed transducers of length and rate of change of length of the muscle, especially during extension, forms a basis for recent findings that spindle information may contribute to position sense (Goodwin, McCloskey & Matthews, 1972).

4.5 SUMMARY

1. Jaw muscle spindle responses in the conscious cat, during normal masticatory movements, resembled those of the passive receptors in their general form. Maximal frequencies occurred during the lengthening phase, whilst during shortening discharge was reduced or abolished.

2. Passive opening of the jaw typically produced a marked increase in spindle firing. The extent of the increase varied between trials, and appeared to be dependent upon the type of motor activity in which the cat was initially engaged.

3. Spindle units could be divided into two groups according to maximal firing frequencies during eating movements. "High frequency" units attained instantaneous frequencies of 220-320 i.p.s. and were velocity sensitive. "Low frequency" units achieved frequencies of 60-160 i.p.s. and were more static in their behaviour. In addition, "high frequency" units were found to be far more sensitive to local muscle pressure. These two groups of units are belived to correspond to "primary" and "secondary" afferents respectively.

4. Evidence of considerable variability in the relationship between α and γ efferent discharge was found. Patterns of fusimotor drive appeared to differ between eating and drinking movements. Occasionally, during other related movements, e.g. licking the lips, fluctuations in fusimotor discharge were such as to maintain spindle frequency almost constant.

5. In this preliminary study no clear evidence of appreciable spindle participation in load compensation could be found.

APPENDIX A

HISTOCHEMICAL CLASSIFICATION OF CAT MUSCLE FIBRE TYPES

This appendix is not intended to provide a comprehensive guide to the histochemical classification of mammalian muscle fibres (for detailed references see Close, 1972), but simply to review fibre typing in cat muscles and to summarize the present situation in this field. In addition, it is hoped to point out certain misunderstandings that have arisen from attempts to generalize from hetero- to homogeneous muscles in this species and to draw attention to interspecies (cat-rat) differences which are often ignored.

Despite an extensive literature on the histochemical classification of muscle fibres in various mammals, e.g. human, rat, guinea pig and rabbit, relatively little work has been done on cat muscles. Furthermore, the principal studies in the cat have been restricted to hindlimb muscles.

The first major histochemical investigation of cat muscle was made by Henneman & Olson (1965) on the medial gastrocnemius and soleus. A single histochemical technique was employed, namely the lead method for mitochondrial ATPase (Wachstein & Meisel, 1957). Fibres were classified according to the overall degree of ATPase staining and the size and distribution of mitochondria.

In medial gastrocnemius three main fibre types were described. These were referred to as "A", "B" and "C", following, it was claimed, the classification of Stein & Padykula (1962) for rat muscle, which was based on mitochondrial distribution. "A" fibres were large, pale, had few mitochondria and were most numerous. "B" fibres had moderate

enzyme activity, a peripheral concentration of mitochondria and were of intermediate size and number. "C" fibres were small, showed intense staining, had many small mitochondria distributed evenly throughout the fibres and were least plentiful.

In contrast, the soleus muscle was composed exclusively of one fibre type. These fibres most closely resembled the "B" fibres of gastrocnemius, having moderate mitochondrial ATPase activity with a slight concentration of mitochondria peripherally.

Figs. A.1 and A.2 show sections of these muscles.

On the evidence of the distribution of mitochondria it was suggested that "B" fibres were common to the heterogeneous pale gastrocnemius and the homogeneous red soleus.

Subsequently Olson & Swett (1966) repeated a similar study on the flexor digitorum longus (FDL) and flexor hallucis longus (FHL). In both muscles three fibre types were found corresponding to the "A", "B" and "C" fibres previously described in gastrocnemius. "A" fibres were the most, and "B" fibres the least, common.

Yellin & Guth (1970) employed two enzyme techniques in a study of the cat and rat tibialis anterior muscles. Mitochondrial distribution was demonstrated by staining for succinic dehydrogenase (SDH) activity. The calcium method (Samaha, Guth & Albers, 1970) for myosin ATPase was also applied.

As in previous studies, the SDH method revealed that three types of fibres could be separated, on the basis of mitochondrial distribution, which resembled those identified in gastrocnemius, FDL and FHL.

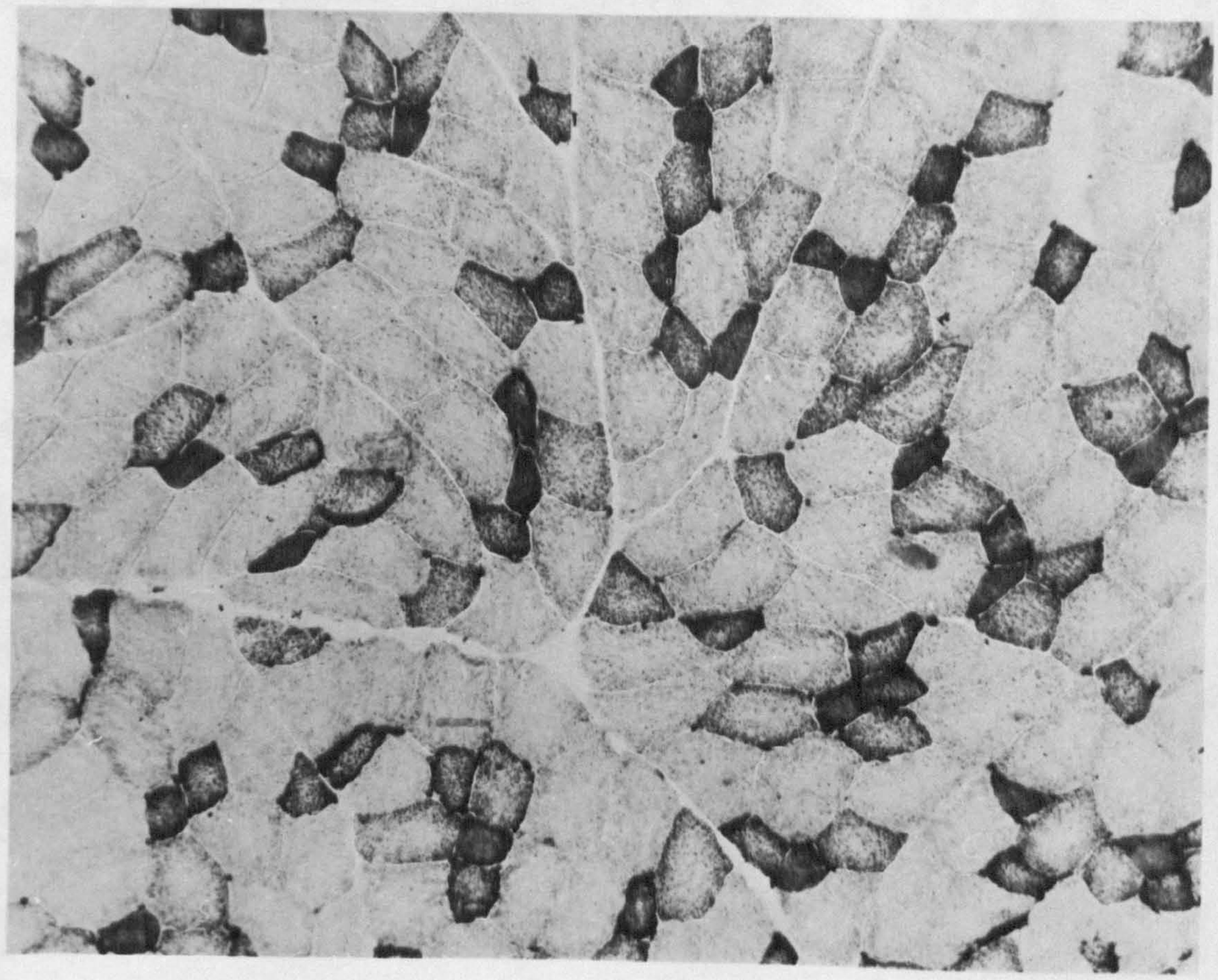


Fig. A.1
Section of cat gastrocnemius muscle stained for mitochondrial ATPase (from Henneman & Olson, 1965).

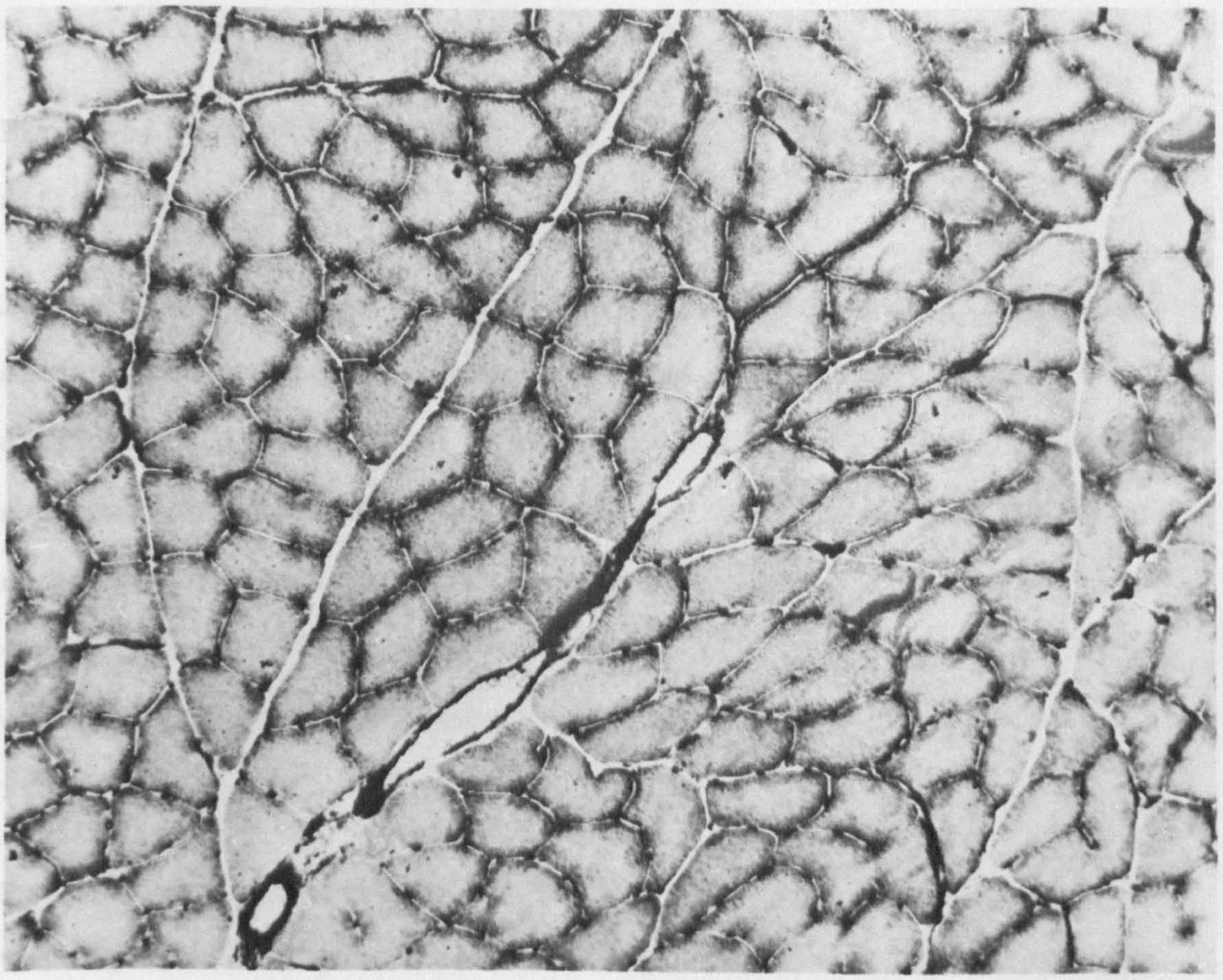


Fig. A.2
Section of cat soleus muscle stained for mitochondrial ATPase (from Henneman & Olson, 1965).

However, the use of MATPase staining, in addition to a mitochondrial enzyme reaction, made apparent certain basic differences between cat and rat muscle fibre types and thereby created difficulties in the definition of a common nomenclature.

Fig. A.3, taken from Yellin & Guth (1970), illustrates these differences.

If the distribution of SDH activity (mitochondrial) is considered alone (corresponding to Henneman & Olson, 1965) there is little difficulty. One group of fibres contains very few mitochondria, a second group contains many large mitochondria, and a third group contains many small mitochondria which are distributed evenly through the cell (with some subsarcolemminal concentration). These would correspond respectively to the "A", "B" and "C" rat muscle fibre types.

Problems arise, however, when the MATPase (alkali preincubation technique) staining of these fibre types is examined. In the rat, "A" fibres show intermediate MATPase activity, whereas in the cat the fibres corresponding to these in their mitochondrial distribution have the strongest reaction. The rat "B" fibres stain most intensely for MATPase whilst in the cat the fibres having a comparable pattern of SDH activity show an intermediate MATPase reaction. Finally, rat "C" fibres show the strongest MATPase staining whereas the cat fibres with a similar mitochondrial arrangement stain very weakly for MATPase.

These discrepancies are summarized in Table A.1.

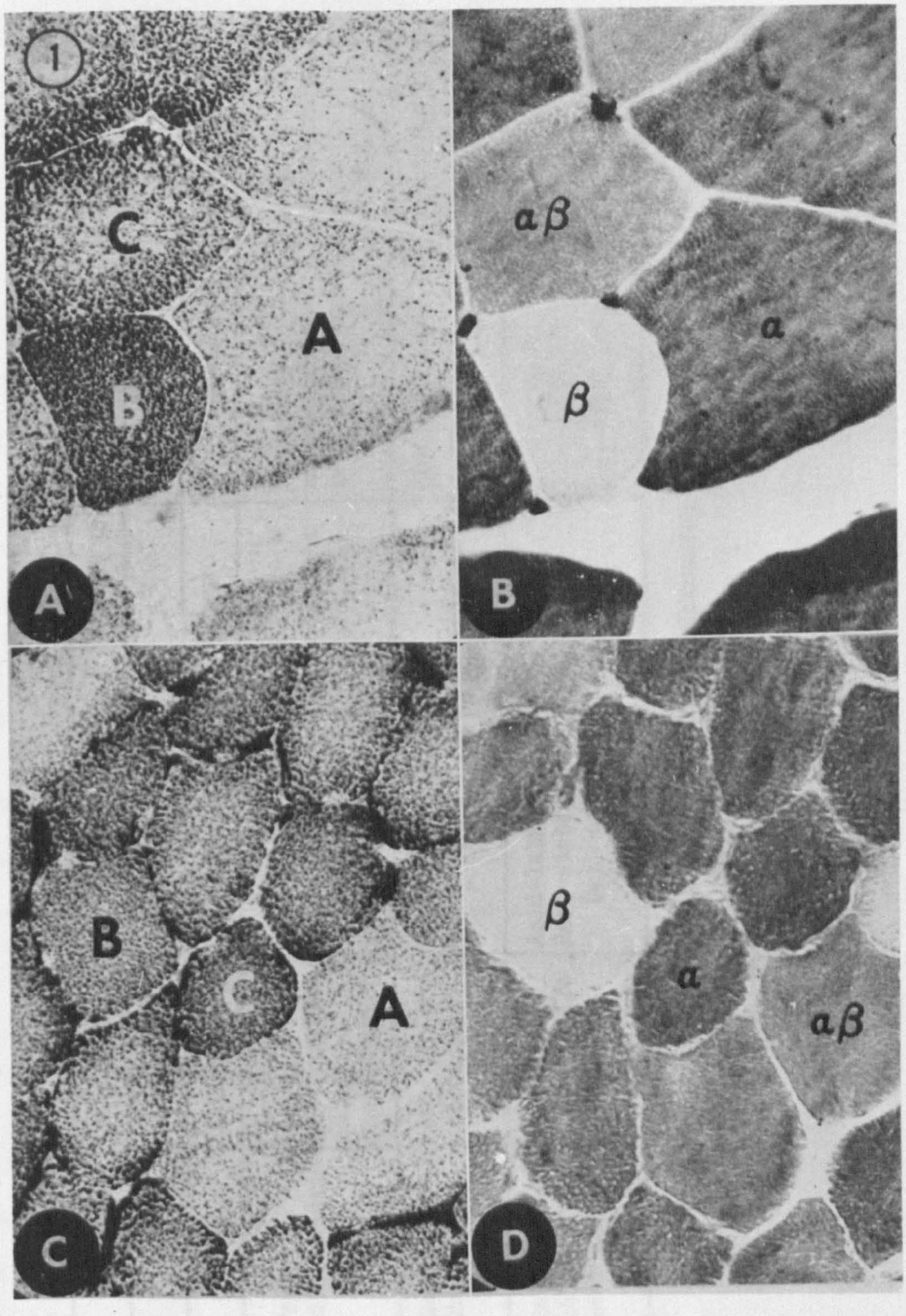


Fig. A.3
Sections of cat (A) and (B) and rat (C) and (D) tibialis anterior. Sections on the left were stained for SDH activity and those on the right for MATPase (from Yellin & Guth, 1970).

TABLE A.1 DIFFERENCES BETWEEN CAT AND RAT HISTOCHEMICAL FIBRE TYPES IN MIXED HINDLIMB MUSCLES.

PROPERTY	RAT			CAT		
SIZE	A*	B	C	A	B	C
	LARGE	INTERMEDIATE	SMALL	LARGE	SMALL	INTERMEDIATE
SDH	Few mitochondria	Large mitochondria, mainly peripheral	Many small mitochondria, (subsarcolemmal) (concentration)	Few mitochondria	Many small mitochondria, even distribution	Large mitochondria, mainly peripheral
MATPase (alkali preincubation)	Intermediate activity	Low activity	High activity	High activity	Low activity	Intermediate activity
MATPase lability	Intermediate lability in acid and base ($\alpha\beta$)	Base-labile Acid-stabile (β)	Acid-labile Base-stabile (α)	(α)	(β)	($\alpha\beta$)

* Yellin & Guth (1970) Classification

On the basis of these differences Yellin & Guth (1970) applied a different classification to that of Henneman & Olson (1965), reversing the fibre types referred to as "B" and "C".

The combination of SDH and MATPase staining also shows up variations between the single fibre types of the cat soleus and the "intermediate" or "C" (Yellin & Guth, 1970) fibres of the heterogeneous limb muscles. Although these are essentially similar according to mitochondrial distribution, they differ in MATPase staining. In the heterogeneous muscles, e.g. gastrocnemius, these fibres have intermediate MATPase activity, whereas, in soleus MATPase staining is low (personal observation). Thus the muscle fibres of cat soleus constitute a quite separate type, a fact that is not always appreciated (see Close, 1972).

In recent years, Burke and coworkers (1971, 1973), in the course of attempting to correlate the physiological and histochemical characteristics of single motor units, have examined cat gastrocnemius with a variety of histochemical methods. The majority of units fell into one or other of three groups according to their contractile properties. FF units appear to correspond to "A" (Yellin & Guth, 1970) fibres, FR units to "C" fibres and S units to "B" fibres.

Ariano, Armstrong & Edgerton (1973) have studied hindlimb muscle fibre populations in five mammals, including the cat. Comparison of the findings of these authors with those of previous workers is complicated by their classification fibres into fast oxidative glycolytic (FOG), fast glycolytic (FG) and slow oxidative (SO). Under this regime fibres in each of the muscles studied, both hetero- and homogeneous, were allocated to one of these three types.

Table A.2 lists the principal histochemical studies on cat muscle, stating the muscles used, the techniques applied and the classification adopted.

TABLE A.2. HISTOCHEMICAL STUDIES ON CAT MUSCLES

AUTHORS	MUSCLE	STAINING TECHNIQUES	CLASSIFICATION *
Henneman & Olson (1965)	Gastrocnemius Soleus	Mitochondrial ATPase Mitochondrial ATPase	A(A), B(C), C(B) B(C?)
Olson & Swett (1966)	Flexor Digitorum Longus Flexor Hallucis Longus	Mitochondrial ATPase Mitochondrial ATPase	A(A), B(C), C(B) A(A), B(C), C(B)
Yellin & Guth (1970)	Tibialis Anterior	SDH MATPase (a) Alkali Preincubation (pH, 10.4) (b) Acid Preincubation (pH, 4.35)	A, B, C
Burke & Others (1973)	Gastrocnemius	PAS (glycogen) Phosphorylase DPNHD[1] SDH MATPase (a) EDTA - ATPase[2] (b) Ac - ATPase[3] Esterase M-α-GPD[4] LDH[5] Neutral Fat	Motor Units Classified FF, FR and S.

* Equivalent Yellin & Guth (1970) Classification in Brackets.

1 Reduced Diphosphopyridine Nucleotide Dehydrogenase.

2 Edetic Acid Buffer (pH 4.35)

3 Veronal Acetate Buffer (pH 4.65)

4 Menadione-Linked α-Glycerophosphate Dehydrogenase

5 Lactate Dehydrogenase

Contd/...

TABLE A.2 (Contd.)

AUTHORS	MUSCLE	STAINING TECHNIQUES	CLASSIFICATION *
Ariano & Others (1973)	Gracilis	MATPase	Fast Oxidative Glycolytic (C)
	Sartorius	DPNHD	Fast Glycolytic (A)
	Tensor Fascia Latae	M-α-GPD	Slow Oxidative (B)
	Tenuissimus		
	Adductor Brevis		
	Adductor Longus		
	Adductor Magnus		
	Pectineus		
	Caudofemoris		
	Cruralis		
	Rectus Femoris		
	Vastus Intermedius		
	Vastus Medialis		
	Vastus Lateralis		
	Biceps Femoris		
	Semi Membranosis		
	Semi Tendinosus		
	Popliteus		
	Gastrocnemius Lat.		
	Gastrocnemius Med.		
	Plantaris		
	Soleus		
	Tibialis Posterior		
	Extensor Digitorum Longus		
	Flexor Digitorum Longus		
	Flexor Hallucis Longus		
	Peroneus Brevis		
	Peroneus Longus		
	Peroneus Tertius		
	Tibialis Anterior		

APPENDIX B.

HISTOCHEMICAL METHODS

All techniques were carried out on frozen sections freshly cut at 10 μm between -15°C and -20°C.

B.1 Calcium activated MATPase (after Padykula & Herman, 1955).

1. ATP fixative.

0.1M	Cacodylate buffer, pH 7.2	90 ml
40%	Methanol free formalin	10 ml
	Sucrose	12.5 g
	Sodium pyrophosphate	125 mg

Adjust to pH 7.4 using 0.1N sodium hydroxide.

2. Incubation medium.

	Adenosine triphosphatase (disodium salt)	15 mg
	Cysteine	15 mg
0.32M	Calcium chloride	1 ml
0.1 M	Sodium barbitone	2 ml
	Distilled water	7 ml

Adjust pH to 9.4 using 0.1N sodium hydroxide.

3. Staining Procedure.

(1) Dry sections in air.

(2) Fix sections at -4°C for 1.5 hr.

(3) Incubate sections at 37°C for 1-3 hr.

(4) Wash sections in three changes of 1% calcium chloride for 10 min.

(5) Transfer sections to 2% cobaltous chloride for 3 min.

(6) Wash sections in several changes of distilled water.

(7) Immerse sections in 1% yellow ammonium sulphide for 2 min.

(8) Wash sections thoroughly in tap water.

(9) Dehydrate sections in 80%, 90% and absolute ethanol.

(10) Mount sections in Xam.

4. Result.

Brown-black staining indicates MATPase activity.

B.2 Succinic Dehydrogenase (after Nachlas, Tsou, De Souza, Cheng & Seligman, 1957).

1. Incubation medium.

Buffered succinate	10 ml*
Aqueous solution to Nitro BT (1 mg/ml)	10 ml

* Equal vols. phosphate buffer (0.2M) pH 7.6 and sodium succinate (0.2M).

2. Staining Procedure.

(1) Incubate sections at 37°C for 5-20 min.

(2) Wash sections in 0.9% saline for 1 min.

(3) Fix sections in 10% formol saline for 10 min.

(4) Rinse sections in 15% alcohol.

(5) Mount sections in glycerine jelly.

3. Result.

Blue diformazan reaction product indicates the site of SDH activity.

B.3 Periodic acid - Schiff technique for glycogen.

Staining Procedure.

(1) Fix sections in 10% formol saline for 20 min.

(2) Bring sections to distilled water.

(3) Oxidise sections in 1% periodic acid for 5 min.

(4) Wash sections in tap water, rinse in distilled water.

(5) Place sections in Schiff reagent* for 20 min.

* Schiff reagent prepared according to De Tomasi (1936).

(6) Rinse sections in 0.5% sodium metabisulphite reducer.

Three rinses each of 1 min.

(7) Wash sections in tap water for 10 min.

(8) Dehydrate sections in graded alcohols.

(9) Clear sections in xylol.

(10) Mount sections in Xam.

Dissolve 1 g basic fuchsin in 200 ml hot distilled water. Cool to 50°C and filter. To filtrate add 20 ml N hydrochloric acid and mix well. Cool to 25°C and add 1 g sodium metabisulphite. Shake well and store in the dark for 16-25 hr. Add 2 g activated charcoal; shake for 1 min and allow to stand for 15 min. Filter and store at 4°C.

Result.

PAS positive material, e.g. glycogen stains magenta.

B.4. Sudan black technique for lipid.

Staining procedure.

(1) Place sections in 70% ethanol for 5 min.

(2) Stain sections in Sudan black for 10 min.

(3) Rinse sections in 70% ethanol.

(4) Wash sections in tap water.

(5) Mount sections in glycerine jelly.

Result.

Lipid stains black.

APPENDIX C.

GLASS COATED TUNGSTEN MICROELECTRODES

The method of production of glass coated tungsten microelectrodes was based on that described by Merrill & Ainsworth (1972).

The technique consists of the following main stages:- (a) etching tungsten wire, (b) glass coating, (c) removal of glass from electrode tip and (d) tip plating.

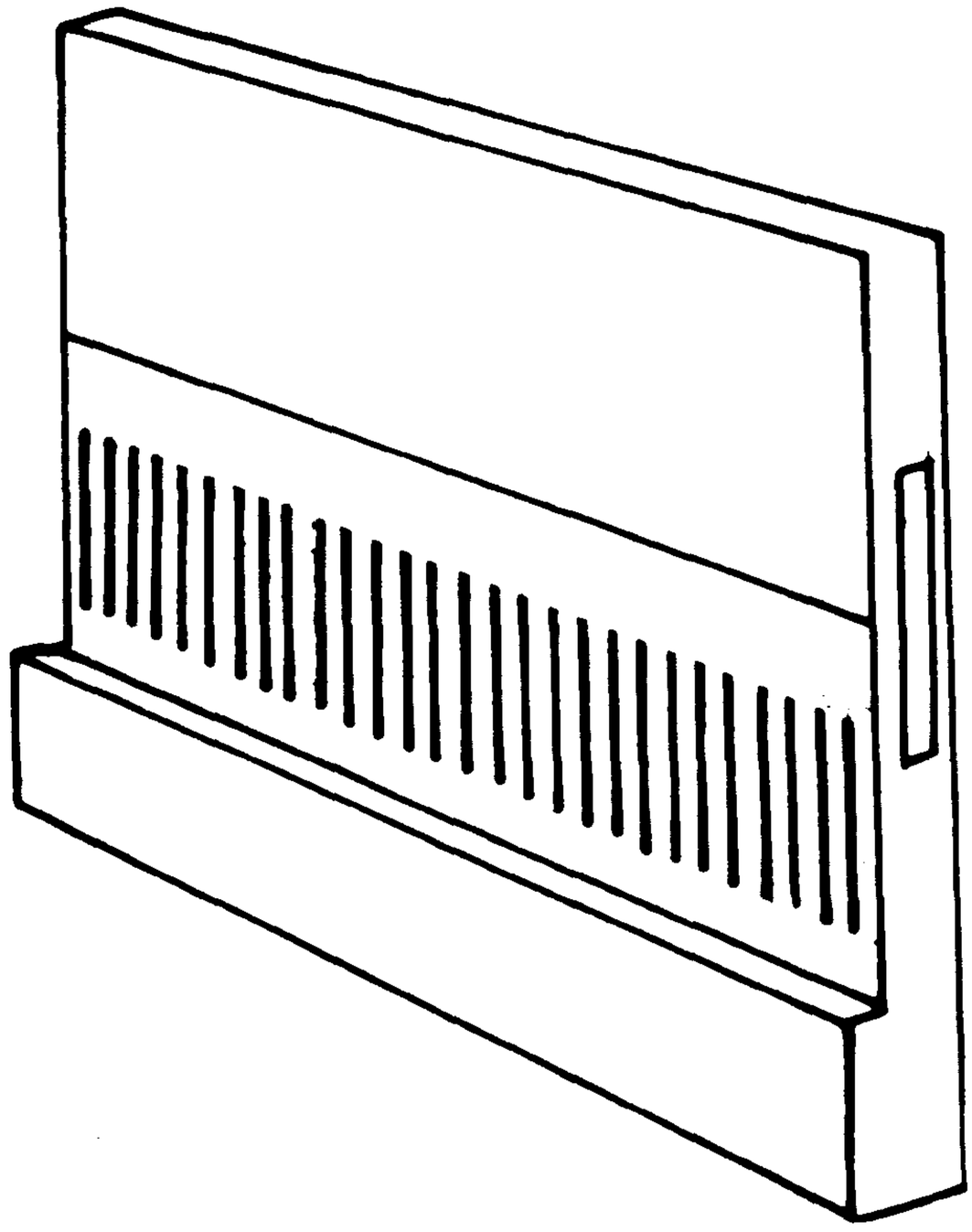
(a) Etching.

Commercially manufactured 4 cm lengths of prestraightened tungsten wire of diamter 127 μm (General Electric Co., Lamp Metals Dept., Ohio, U.S.A.) were used.

Wires were aligned in a jig (Fig.C.1(a)) consisting of thirty pieces of hyperdermic-needle tubing cemented in parallel to a plastic plate. A stop ensured that the ends of individual wires were lined up. A strip of adhesive tape was placed across the wires which were then lifted free. The tape bearing the wires was wound around a brass spindle (Fig.C.1(b)). The circumference of the spindle was such that the wires were equally spaced, allowing uniform etching.

The wires were etched in a fresh solution of potassium nitrite (KNO_2, 150 g per 100 ml distilled water) at room temperature. The etching voltage (6v) was supplied by a step down transformer and variable transformer. Etching voltage was monitored by an a.c. voltmeter. Electrical connexion was made to the spindle and to a large carbon rod (10 cm long by 1 cm diameter) placed in one corner of the etching bath (Fig. C.2). The spindle was mounted vertically in a micromanipulator and wires advanced sinusoidally (1 Hz) into the

a



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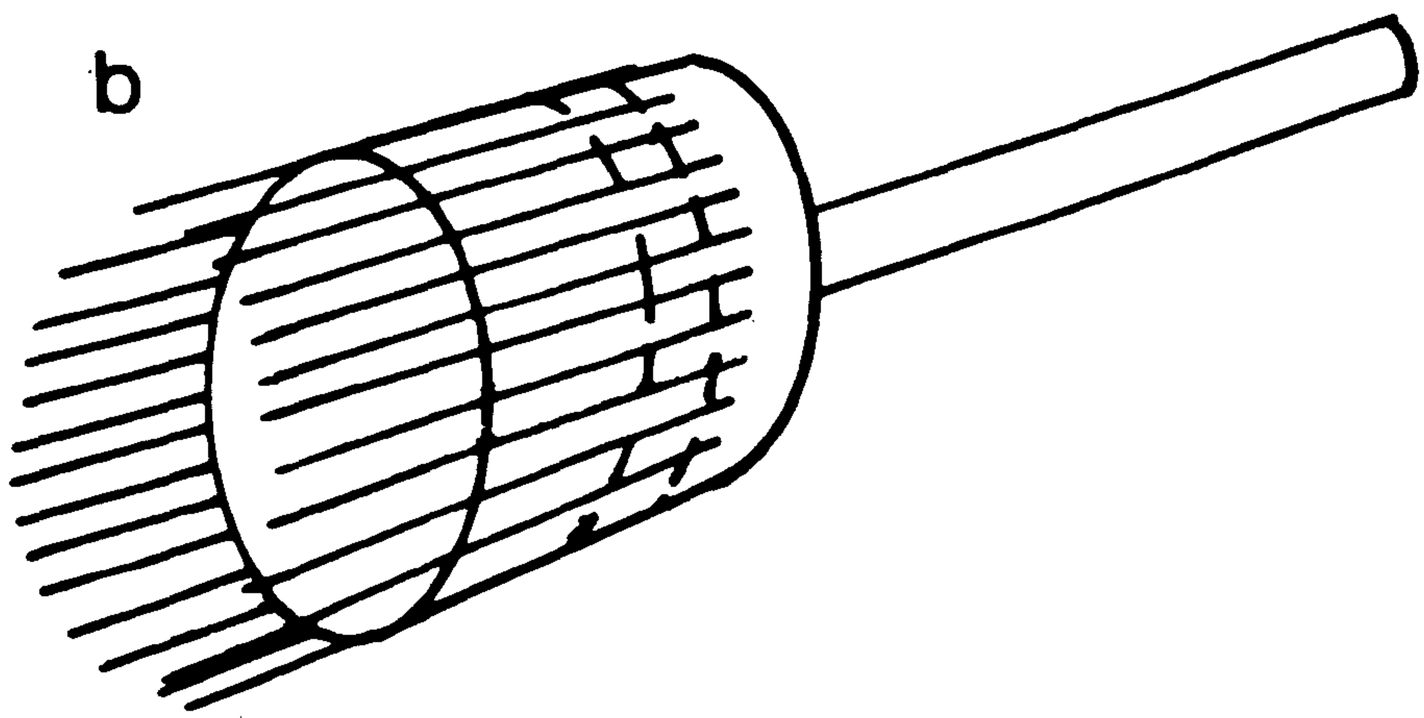

Fig. C.1
(a) Jig for the alignment of tungsten wires.
(b) Tungsten wires taped to the spindle for etching.

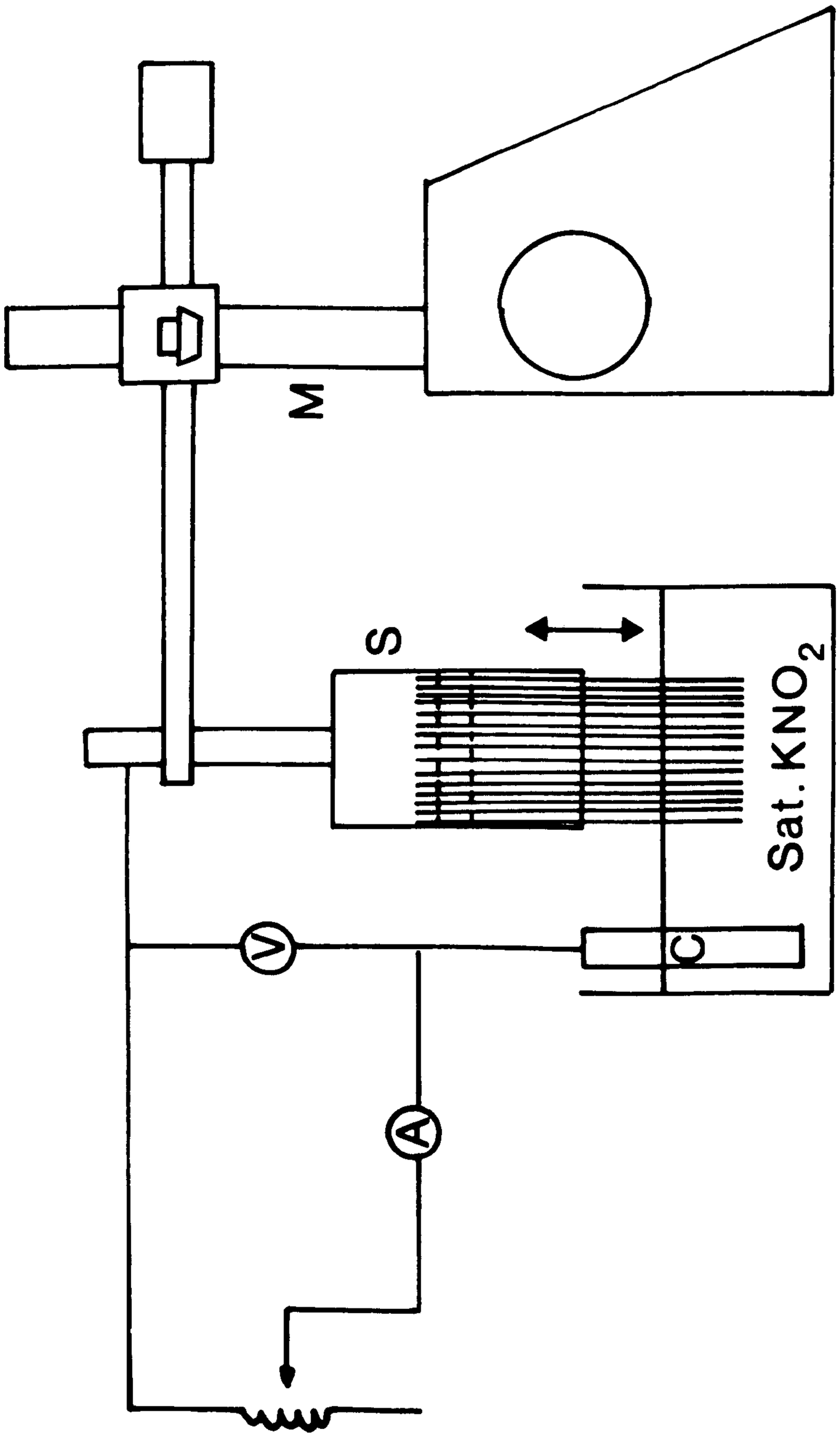

Fig.C.2 Etching apparatus.
Tungsten wires are taped to the spindle (S) and their tips sinuisoidally immersed in the etching solution using the micromanipulator (M). AC for etching is applied between the shaft of the spindle and the large carbon rod (C).

solution to a depth of 1.0 cm. Etching takes place during the positive half of the cycle.

Periods of etching of 4.5 min (at 6v) were found to produce tips of uniform taper of approximately 5^{o} over the terminal region. Such tip profiles were sufficiently fine in relation to neuronal dimensions and also provided adequate mechanical strength.

(b) Glass coating.

Electrodes were insulated by collapsing borosilicate capillary tubing (Jencons (Scientific), O.D. 2 mm, I.D. 1.0 mm) on to etched wire using a horizontal glass micropipette puller (Scientific & Research Instruments Ltd.).

Tubing was cut into lengths of 7 cm, and cleaned by boiling in detergent solution and distilled water, rinsing in acetone and allowing to dry.

The etched wire is inserted into the tubing, butt end first, and allowed to slide down until it occupied a central position. The tube is then arranged in the chucks of the puller so that about 3 mm of the butt end of the wire extends beyond the heating coil in the direction to which the tube is to be pulled. Current is passed through the heating element using a variable mains transformer and a step down transformer. Sufficient heat must be applied to the glass tubing to cause it to soften. Gentle manual pulling causes the glass to constrict over the wire and to hold it firmly. The rest of the wire is pulled, at increasing speed through the molten constriction (Fig. C.3).

Coil temperature and force and rate of pull are critical. If coil temperature is too low or the pull too rapid the glass breaks off

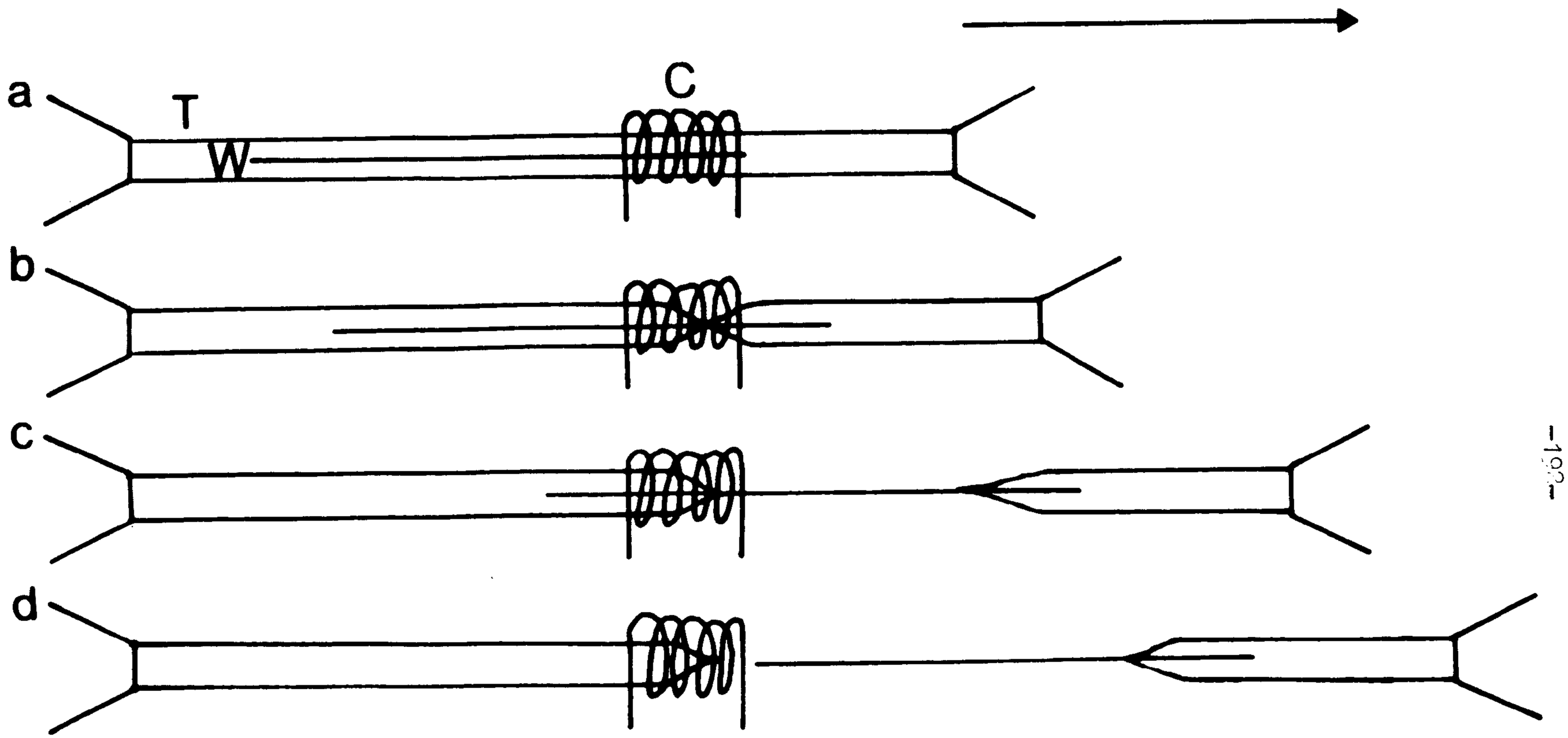

Fig.C.3

(a) The tungsten wire (W) is inserted into the glass capillary tubing (T) and positioned with the butt end just beyond the heating coil (C).

(b), (c) and (d) Current is passed through C and the wire pulled through the molten constriction producing a thin glass coat.

short of the tip. If the temperature is too high or the pull too slow the tube will not collapse completely.

The ideal combination of these parameters results in a thin even coating of the wire, following the taper and extending just beyond the tip.

The coated wire may then be removed from the glass tubing by breaking the insulated near the butt end with a pair of forceps.

(c) Removal of glass from electrode tip.

The desired amount of glass may be removed by inserting the electrode into a heated solder glass (Corning, 7570) bead, allowing the solder glass to cool and then withdrawing the electrode. This results in a clean fracture of the insulating glass at its junction with the bead.

The process is carried out under the microscope (150 x) with a graduated eye piece. A drop of solder glass is held in a platinum heating loop mounted on the moveable stage of the microscope. Current through the loop is variable so that the glass is soft but not molten (will dimple on inserting electrode).

The electrode is held in a pin vice and micromanipulator (3 dimensions, Prior, Ltd.) and advanced into the bead to the level at which it is desired that the insulation terminate (Fig. C.4). Coil heat is switched off and the bead allowed to cool. The electrode may then be withdrawn. Since dimpling of the softened bead's surface tends to obscure the true length of electrode enclosed by solder glass it is helpful to pull the bead away from the electrode until the junction is revealed.

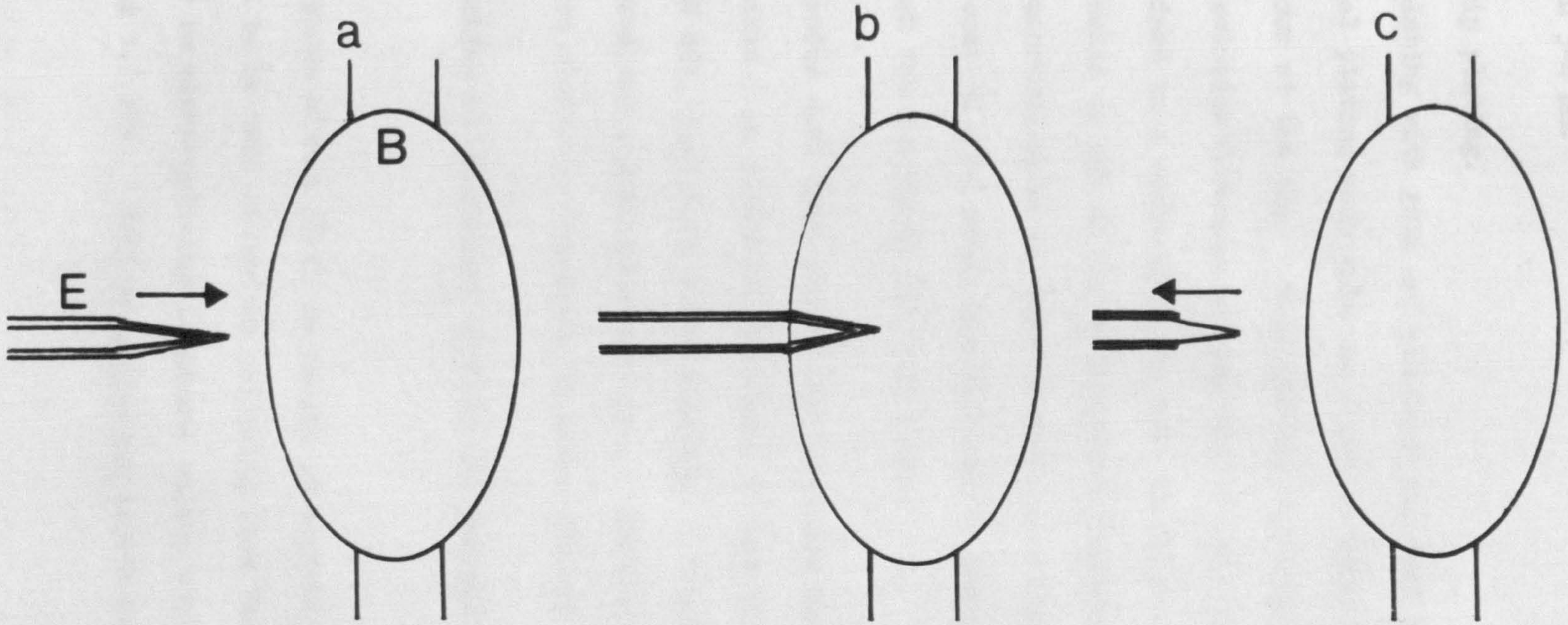

Fig. C.4

(a) The electrode (E) is initially advanced towards the heated solder glass bead (B).
(b) The electrode is inserted into the bead so that the electrode-bead interface coincides with the point at which the glass coating is required to terminate. The bead is allowed to cool.
(c) The electrode is withdrawn leaving the tungsten tip exposed.

Using this technique the exposed tip length may be controlled to within 3-4 μm.

(d) Tip plating.

Tip plating with gold and platinum improved recording characteristics. Initial plating with gold was found to enhance the retention of platinum at the tip. Gold plating was done using a commercial gold salt solution (Johnson Matthey Metals Ltd., G40). This solution was contained in a vertical glass tube in which a loop of platinum wire was sealed to act as the indifferent electrode. Electrodes, held in a micromanipulator, were inserted to a depth of 7 mm. A thin even coat of gold metal was produced by passing a current of 5×10^{-8}A through the electrode for some 5 sec.

Electrodes were then coated with platinum black. The plating solution contained 3 gm platinous chloride, 25 mgm of lead acetate in 100 ml 0.025N HCl, plus 0.05 gm of gelatine. Passing 3×10^{-7}A for 10-20 sec produced a good adherent coat. Plating in this manner routinely reduced electrode impedance to below 1MΩ at 1.7 kHz.

Properties of electrodes used in the present study.

Electrodes with a 20-25 μm length of exposed tungsten of 5° taper were found to be well suited to recording from the large MeNV cells. Prior to electroplating impedance values would be in the range 8-12 MΩ at 1.7 kHz. Following plating, impedances were < 1 MΩ.

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Classification of jaw muscle spindle afferents in the cat

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Much of our knowledge of muscle spindles comes from studies of hind-limb muscles of the cat. Histologically there appear to be approximately equal numbers of primary and secondary spindle afferents. Dorsal-root filament recordings have not been made which would permit this to be statistically corroborated by physiological evidence. However, in the case of the jaw muscles the first order afferent cell bodies, being situated in the mesencephalic nucleus of the trigeminal nerve (mid-n V) as shown by Corbin & Harrison (1940), are accessible to sampling by extracellular micro-electrodes in a way less likely to be biased according to fibre diameter.

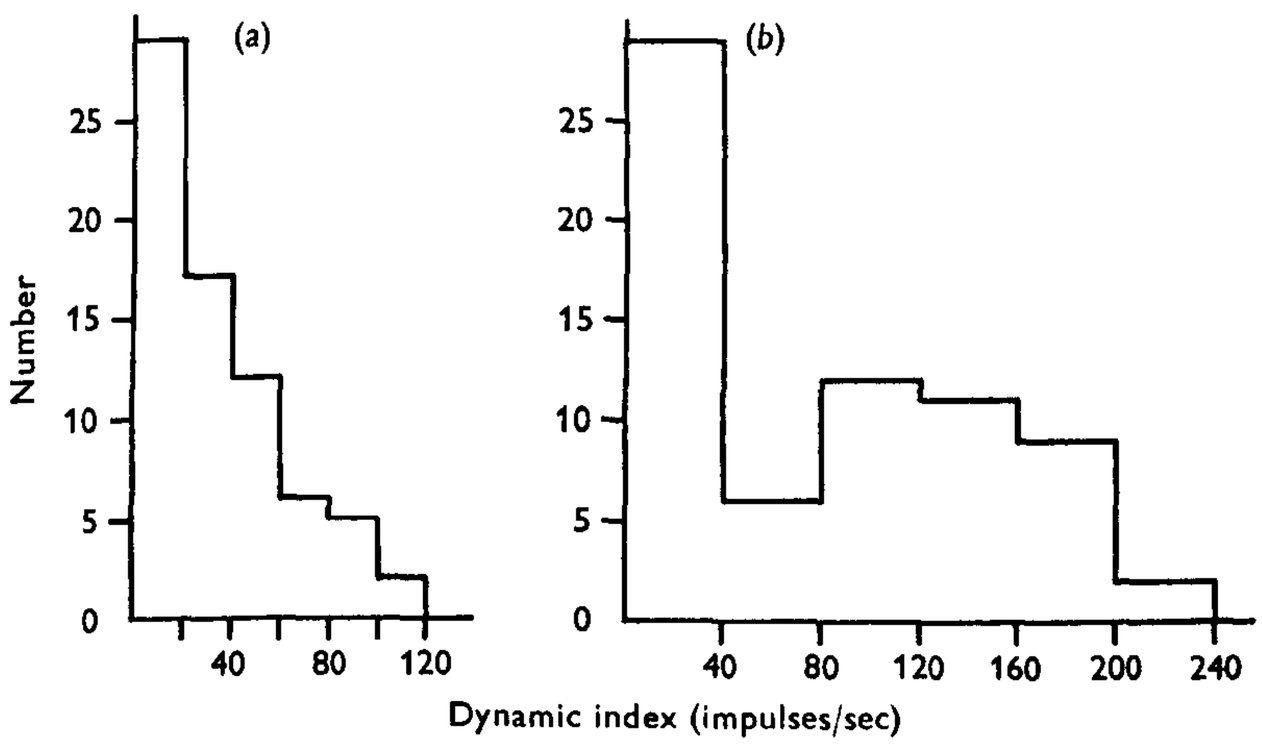

Fig. 1. Histograms of DI from a group of seventy units each tested before (*a*) and 1 min after (*b*) SCh (200 μg/kg I.V.) at ramp velocity of jaw opening 4·5 degrees/sec.

Using the methods described by Davey & Taylor (1966), units characterized as spindle afferents were examined for dynamic index (DI) of Crowe & Matthews (1964) using ramps (1·5 degrees of jaw opening from 8·5 degrees, at velocities of 1·0, 2·2, 3·25 and 4·5 degrees/sec), for small amplitude vibration driving, and for interspike interval variability. Fusimotor activity was suppressed by deep pentobarbitone anaesthesia supplemented by chlorpromazine.

Under these conditions a histogram of DI gave no evidence of two distinct populations of afferents but was indistinguishable from a log-

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normal distribution. In addition, neither the maximum vibration frequency followed (Brown, Engberg & Matthews, 1967) nor the coefficient of variation (Stein & Matthews, 1965) allowed separation into two populations. However, the administration of suxamethonium (SCh) caused a marked change in the histogram of DI. It appears that some units with a previously low DI are capable, when activated with SCh, of a considerable dynamic response, and following the work of Rack & Westbury (1966) should probably be classed as primaries.

It is concluded that the spindle afferents of the jaw muscles are functionally divisible into primary and secondary populations, in proportions similar to those of the limb muscles.

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A FUNCTIONAL ANALYSIS OF THE COMPONENTS OF THE MESENCEPHALIC NUCLEUS OF THE FIFTH NERVE IN THE CAT

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SUMMARY

1. The mesencephalic nucleus of the trigeminal nerve has been studied using extracellular micro-electrode recording and the constituent cell types identified.

2. Two types of unit were found, namely, muscle spindle first order afferents of ipsilateral jaw-closing muscles and mechanoreceptor afferents of ipsilateral maxillary and mandibular teeth.

3. No evidence was found for representation of extra-ocular muscle stretch receptors, of temporo-mandibular joint receptors or of tendon organs of jaw muscles.

4. Spindle units of each of the jaw-closing muscles were recorded in all parts of the nucleus and there was no evidence of their segregation according to muscle of origin.

5. Attempts to classify spindle units by their dynamic response to ramp stretches, their following of high frequency vibration and their interspike interval variability at constant length gave no indication of two populations when fusimotor activity was suppressed.

6. Following the injection of suxamethonium, however, units fell into two groups according to their dynamic index. Their behaviour resembled that described for primary and secondary spindle afferents. In data pooled from all of the jaw-closing muscles there were approximately equal numbers of units in each group.

INTRODUCTION

Much of our knowledge of muscle spindles comes from studies of the hind-limb muscles of the cat, which have contraction speeds (time to peak twitch) of 27–70 msec (Buller, Eccles & Eccles, 1960). One of the purposes

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of the present work was to examine the properties of spindles in the very fast jaw-closing muscles, which are known to be able to reach peak twitch tension in 11 msec (Taylor & Davey, 1968).

The mesencephalic nucleus of the fifth cranial nerve (MeNV) is a collection of cells morphologically similar to dorsal root ganglion cells and believed to be primary or first order neurones from proprioceptors in the cranial region (Cajal, 1909; Hosokawa, 1961). In particular, muscle spindle afferents of the jaw-closing muscles (masseter, temporalis and pterygoid) appear to be represented and are accessible to extracellular micro-electrode recording (Jerge, 1963). Sampling spindle afferents by this means is perhaps less likely to be biased according to fibre diameter than in the case of dorsal root filament recording. This is important in determining whether primary and secondary afferents form two distinct groups separable by physiological means, or are a single continuous population. Evidence for such a division has been presented for the hind-limb muscles (Matthews, 1963), but classification has been unsuccessful in other situations (Koeze, 1968; Bach-y-Rita & Ito, 1966).

In addition, it was hoped to resolve some inconsistencies in the findings of previous workers concerning the cell types present in the MeNV. The original physiological observations on the nucleus and its root (Pfaffman, 1939; Corbin & Harrison, 1940) indicated that units associated with spindles of the masticatory muscles and periodontal receptors were represented, and this has been confirmed (Jerge, 1963; Taylor & Davey, 1968; Cody, Lee & Taylor, 1972). Smith (1969) claimed that tendon organ cell bodies were also present, though the evidence of Szentagothai (1948) is against this and suggests their presence in the trigeminal ganglion. Much uncertainty also surrounds the question of the location of eye muscle proprioceptor first order cells (Hosokawa, 1961; Whitteridge, 1960). A widely held view is that they also are present in the MeNV (Cooper & Fillenz, 1955; Fillenz, 1955). On the other hand, evidence from the pig and sheep (Manni, Bortolami & Desole, 1966, 1968) places them in the trigeminal ganglion. Recordings from more than five hundred single units in the nucleus have permitted us to check some of these possibilities in the cat.

METHODS

Adult cats, male and female, in the weight range 2–3 kg were used. They were anaesthetized with sodium pentobarbitone (60 mg/kg I.P.) and maintained at a deep level by I.V. supplements. In later experiments, while recording from muscle spindles, chlorpromazine (I.V. doses of 5 mg half hourly) was used in addition to suppress fusimotor activity. Such doses of chlorpromazine are greater than those shown by Henatsch & Ingvar (1956) to abolish spontaneous and reflexly evoked fusimotor discharge, particularly in combination with pentobarbitone which Voorhoeve & van Kanten (1962) have shown to have similar, though less specific, action.

The animal's head was held in a stereotaxic device (La Précision Cinématographique, for visual experiments) and electrodes inserted vertically through a hole in the cranium, usually with the superior colliculus exposed by hemispherectomy. Access to the more caudal regions of the MeNV was provided by drilling away part of the tentorium.

Recording. Glass-coated tungsten micro-electrodes (Merrill & Ainsworth, 1972) were used, with impedance 1–3 MΩ at 1·7 kHz. Action potentials triggered an instantaneous frequency display circuit.

The whole extent of the MeNV, as determined by histology, was explored. The region of interest is a rostro-caudal strip approximately 1 mm wide and 8 mm in length, centred on the mid-point of the superior colliculus and 2·3 mm from the midline. Electrode tracks were generally spaced at 200 μ intervals. Thirty-nine animals were used and the total number of electrode tracks was seven hundred and seventy-nine.

Application of muscle stretch. The mandible was secured to a light V-shaped frame pivoted about an axis through the temporo-mandibular joints. The apex of the V was coupled to an electromagnetic displacement servo (Pye–Ling V 50 vibrator). The stimuli were ramps of 1·5 degrees of jaw opening starting from 8·5 degrees at velocities of 1·0, 2·2, 3·25, 4·5 and 10·0°/sec. Small amplitude vibrations were also applied at increasing frequencies up to 300 Hz. The maximum frequency at which a receptor could reliably give one impulse per cycle is referred to as the following frequency. Stretch of extra-ocular muscles was produced by passive rotation of the eyeball using a suction cup and stem.

Stimulation of muscles. Pairs of enamelled wires with their final 2 mm bared were inserted into each of the jaw-closing muscles. The pterygoid was reached through the palate.

Histology. In many cases the animals were perfused after death with saline followed by formol saline and the brains subsequently examined for the location of the electrode tracks. Detailed histological studies will be reported elsewhere.

RESULTS

During the exploration of the mid-brain, ramp stretches were continuously applied to the jaw with a cycle period of 2 sec. When the region of the dorsolateral aspect of the central grey matter was approached unitary activity was detected in time with opening movement. Thereafter as the electrode was advanced to isolate a single unit, tests for tooth receptors and eye muscle receptors were frequently applied, together with occasional testing for other sensory input from the head. The only activity detected in this region, in these deeply anaesthetized animals, was related to jaw opening or to tooth pressure. Occasionally, a unit was encountered which was sensitive to eye movement, but invariably it proved not to be specific to direction of rotation. Also, such units were more affected by eyeball pressure (which would relax most of the extrinsic eye muscles) than by traction. They were always, in addition, extremely sensitive to jaw opening and to local pressure on particular jaw muscles. In recording from more than 500 units, we have never found one specifically related to eye movement.

Cells responding to pressure on teeth were encountered, as described by Jerge (1963). They were most commonly associated with mandibular or maxillary canines. Some were quite specific with regard to direction of pressure on a single tooth, while others could be excited by pressure on several teeth or surrounding gum. They were less plentiful than units responding to jaw opening, but showed no sign of being specifically segregated from them. No further attention was directed to these tooth receptors.

Turning to the cells related to jaw movement, they were always excited by jaw opening, never by closing. They could therefore only have been connected with stretch receptors in the jaw-closing muscles (temporalis, masseter, pterygoid) or possibly with temporo-mandibular joint receptors. The latter possibility is unlikely in view of their unidirectional sensitivity. Furthermore, they could always be activated by light local pressure on one or other of the above three muscles, with the jaw held still. In all cases, the possibility of joint receptors was eliminated in these ways and by the more specific positive tests for muscle spindles, described below.

Before proceeding to analyse the functional characteristics of the muscle stretch receptors, it is worth considering the evidence for the cells concerned being first order. The cells regarded as constituting the MeNV (Cajal, 1909) are large unipolar cells scattered thinly over the dorsolateral surface of the central grey. They are morphologically very similar to dorsal root ganglion cells and show chromatolysis on cutting the root of the fifth nerve (May & Horsley, 1910). Our recordings were always from the region in which these cells were to be found histologically. The unitary activity was very resistant to anaesthesia and could persist for some minutes after respiration had been arrested with pentobarbitone. It was also notable that the cells very seldom showed signs of injury by repeated close passage of the electrode tip. This is consistent with the cells having no dendritic tree. In those few cases in which other, unrelated, activity was noticed in this region, the jaw-opening units generally gave larger extracellular action potentials, as expected from the large size of first order somata in the MeNV. Latency measurements were not attempted in these experiments because of the difficulty of exposing the relevant nerves without destroying large parts of the muscles, and because the conduction distances are so uncertain.

Distinction of cells belonging to muscle spindles and tendon organs

On the basis of their responses to muscle twitches, most of the jaw-closing muscle stretch receptors could be immediately classified as belonging to muscle spindles. Some typical records are shown in Fig. 1. The large artifacts at the beginning of the records due to synchronous muscle

action potential could not be avoided, but they do not obscure the spindle type responses of the units, i.e. cessation of discharge during the rising phase and a burst during the falling phase of contraction. Occasionally, contraction of one of the jaw muscles resulted in a discharge during the rising phase of the twitch, suggesting a tendon organ in that muscle.

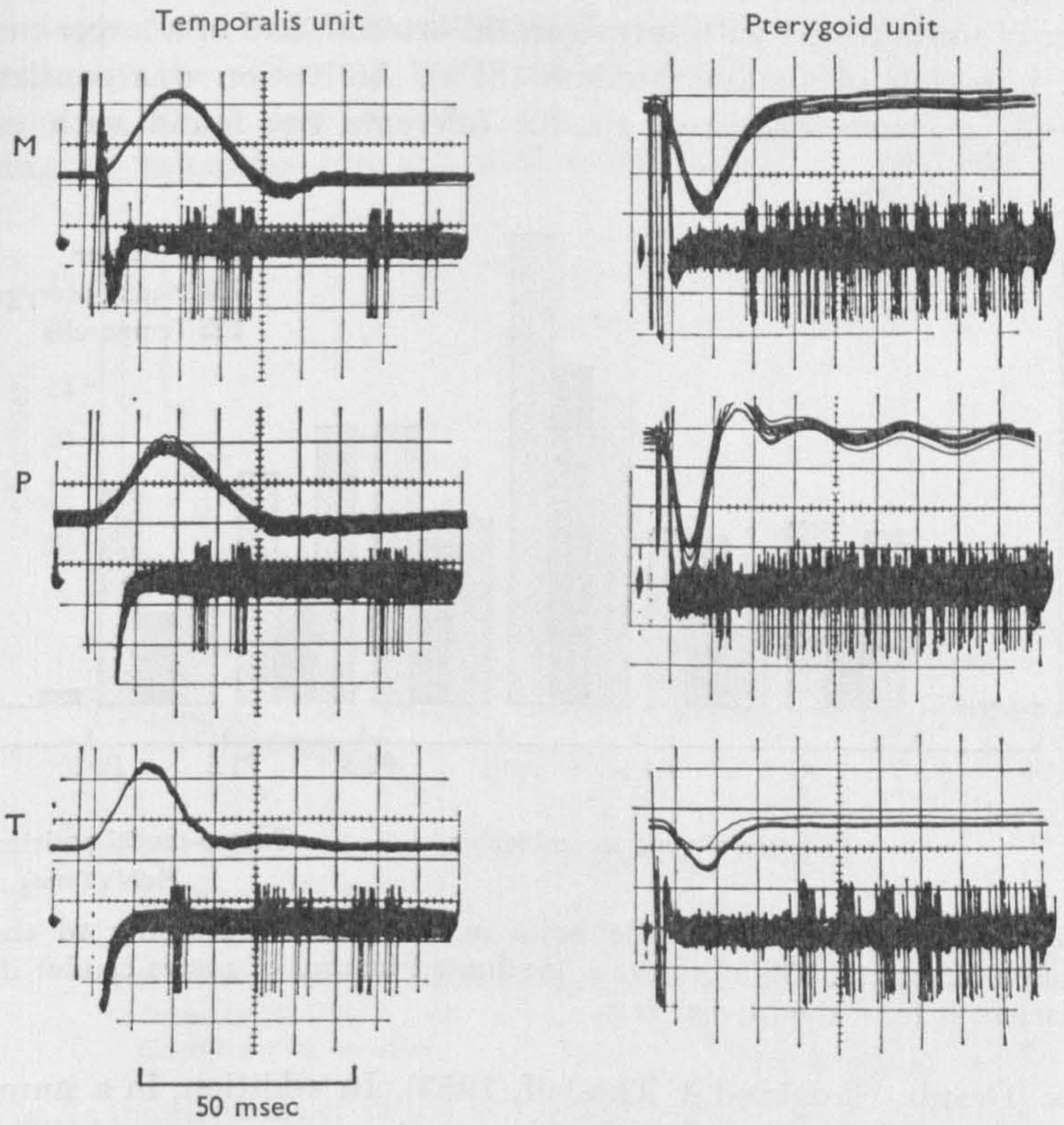

Fig. 1. Responses of two units in the MeNV to twitches of masseter (M), pterygoid (P) and temporalis (T). In each case ten responses are superimposed. The upper trace represents displacement of the jaw. Jaw closing is upward for the temporalis unit (left) and downward for the pterygoid unit (right).

However, closer examination always revealed that the muscle being stimulated was not that in which the receptor was located. The true muscle of origin of the afferent was being stretched by the twitch in its neighbour. Although the three muscles are essentially in parallel as regards jaw opening and closing movements, they can have opposing effects in lateral sliding motion at the temporo-mandibular joint. For example, contraction of masseter not only closes the jaw, but also deflects it laterally and can

stretch pterygoid. In the work reported by Taylor & Davey (1968), in which tendon organ cell bodies were believed to be occasionally found, the problems of this mechanical situation had not been fully appreciated. Study of many more units has now made it clear that all the jaw movement sensitive cells in the MeNV can be accounted for as belonging to spindles in jaw-closing muscles. This was supported by the response of a limited number of units tested with intra-carotid arterial, and of a larger number to I.V. injection of suxamethonium (SCh). Activation was consistently produced, a characteristic of spindle afferents not found with tendon

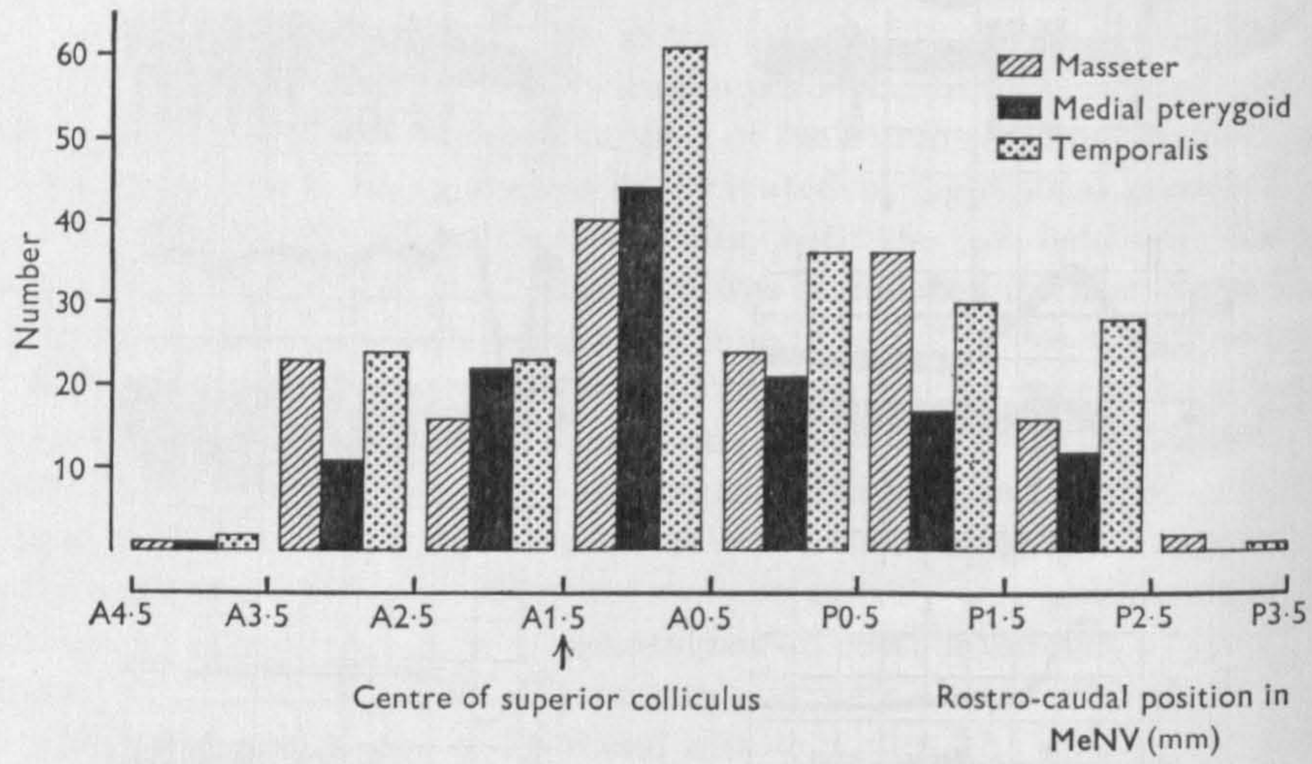

Fig. 2. Distribution of spindle units in the MeNV according to their muscle of origin. The abscissa is graduated in mm of rostro-caudal displacement from the ear-bar zero.

organs (Granit, Skoglund & Thesleff, 1953). In addition, in a number of preliminary experiments jaw-opening units were examined in lightly anaesthetized cats. Under these conditions such units always showed conspicuous frequency changes with pinna twisting (Granit, Job & Kaada, 1952).

Distribution of spindle units according to muscle of origin

The muscle of origin of cell bodies of spindle afferents was identified by (*a*) application of surface pressure to each muscle, (*b*) electrical stimulation of each muscle, (*c*) manipulation of the mandible and (*d*) pressure on the eyeball. Muscle pressure normally allowed reasonably certain localization, one muscle being much more sensitive to probing than the others. In addition, lateral deflexion of the jaw preferentially stretches pterygoid and medial deflexion stretches masseter, and to a lesser extent, temporalis.

Units from each of the jaw-closing muscles were found in all regions of the nucleus (Fig. 2). Applying the χ^2 test, no differences in relative distribution of units from the three muscles could be detected. No attempt was made to examine the medio-lateral distribution because of the narrowness of the nucleus.

Classification of spindle units

Attempts were made to distinguish primary and secondary spindle afferents by the following tests: (*a*) dynamic index (DI) of Crowe & Matthews (1964). Jaw opening was of amplitude 1·5° from 8·5° of opening at velocities 1·0, 2·2, 3·25 and 4·5°/sec. A quotient was also

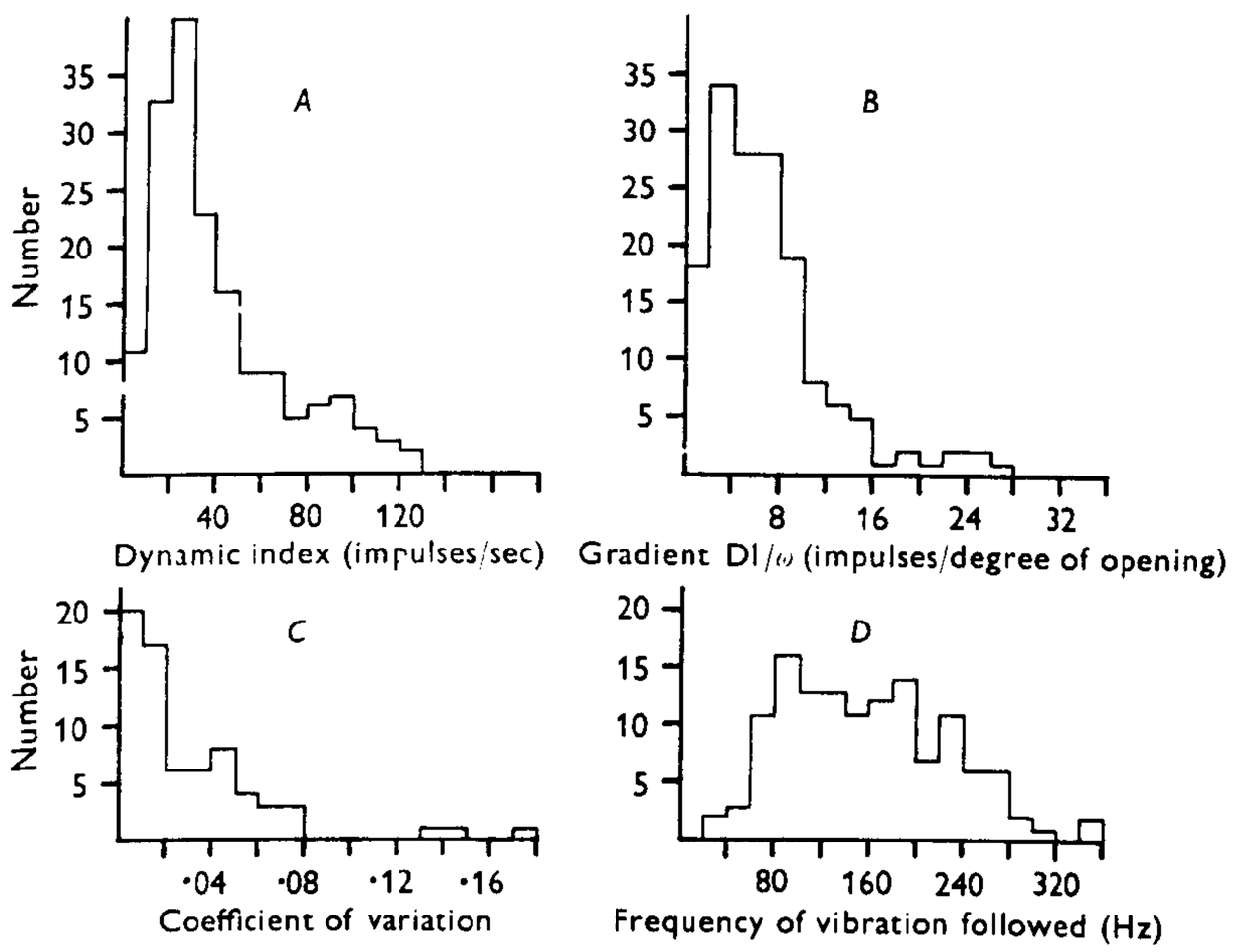

Fig. 3. Distributions of values of (*A*) dynamic index, (*B*) gradient of DI with respect to angular velocity of jaw opening (ω), (*C*) coefficient of variation of impulse intervals at constant muscle length and (*D*) maximal frequency of vibration followed. In all cases fusimotor activity was suppressed by pentobarbitone and chlorpromazine.

derived of DI/velocity by linear regression, and is referred to as 'normalized DI'; (*b*) maximal frequency following during small amplitude sinusoidal stretching at increasing frequencies up to 300 Hz (Brown, Engeberg & Matthews, 1967); (*c*) interspike interval variability (coefficient of variation, CV) during constant maintained stretch (Stein & Matthews, 1965). Responses were always recorded under deep pentobarbitone anaesthesia supplemented by chlorpromazine to suppress fusimotor activity.

The distributions of values of these parameters are plotted in Fig. 3. None of the tests showed a clear separation of units into two groups corresponding to primary and secondary units. Statistical testing showed that the distribution of DI, normalized DI, frequency following and CV were not acceptable as Gaussian but were indistinguishable from lognormal. The histograms of both DI and CV do, however, give some indication of small second peaks. Though these are probably not significant in themselves, the second peak in CV happens to correspond with that found for primary endings by Stein & Matthews (1965).

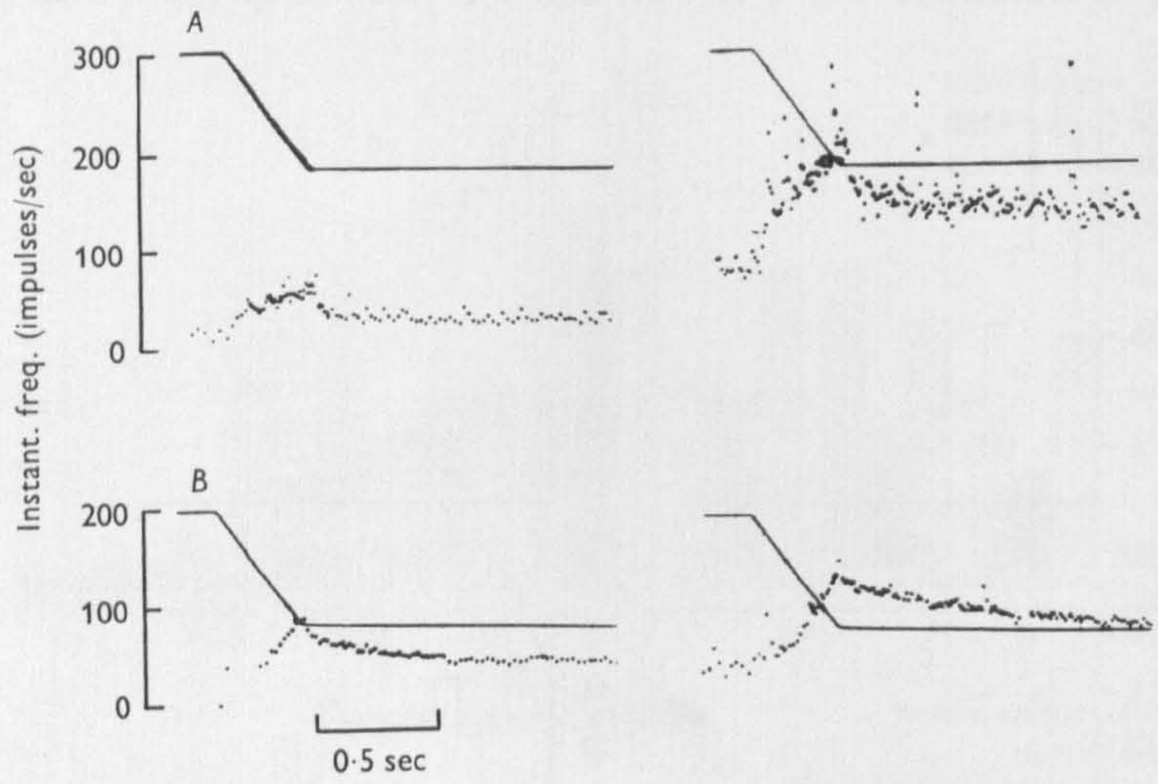

Fig. 4. Responses of the two units, (*A*) pterygoid and (*B*) temporalis, illustrated in Fig. 1 to ramps of jaw opening of 1·5 degrees amplitude. In each case the response on the left is before, and on the right 1 min after, the I.V. administration of 200 μg/kg SCh.

The use of suxamethonium in the classification of spindle units

It was thought that the lack of separation of units into two populations may have been due to many primary units not showing their expected dynamic response in the absence of fusimotor drive. Consequently, in another series of experiments the effect of SCh was tried because it is believed to cause intrafusal contraction especially of nuclear bag fibres, is known to excite primary endings more than secondaries (Fehr, 1965) and has been used previously (Rack & Westbury, 1966) to help classify spindle afferents.

The instantaneous frequency of units was recorded in response to ramps at 1·0, 4·5 and 10·0°/sec, before and after 200 μg/kg SCh I.V. The ramps were timed to begin at respectively 45, 60 and 75 sec after injection.

Artificial ventilation was maintained throughout. SCh administration was followed by an initial reduction or abolition of unitary activity for 5–10 sec. Over the next 30 sec discharge increased irregularly, often being unrelated to muscle extension. Thereafter, the pattern of firing stabilized and correlated with jaw movements. Activation was most consistent at 1 min and subsequently declined to normal at 5–10 min. Comparison of responses before and after SCh was made on the intermediate speed ramp at 1 min.

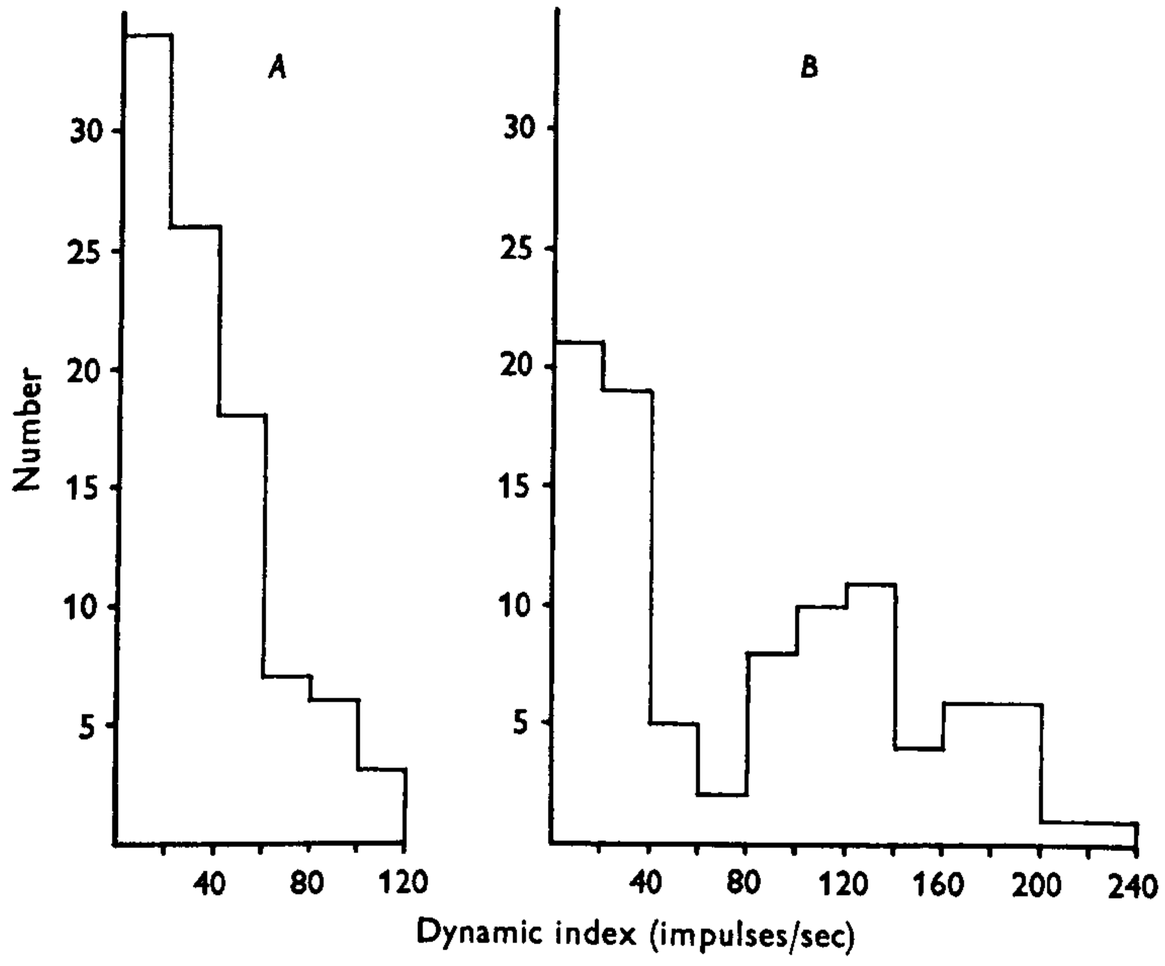

Fig. 5. Distribution of values of DI in ninety-four spindle afferent units (*A*) before, and (*B*) 1 min after 200 μg/kg SCh.

Fig. 4 illustrates the effect of SCh on two units. Initially the DI of both units was similar. After SCh the resting discharge frequency increased. One unit (*A*) showed a large increase in DI and the irregularity of its firing. In contrast there was only a small change in the dynamic response of the other unit (*B*) and its discharge remained regular. These responses resemble those described by Rack & Westbury (1966) for primary and secondary afferents respectively. Histograms of DI for ninety-four units tested in this way at 4·5°/sec are shown in Fig. 5. SCh is seen to convert the single skew (lognormal) distribution into a clearly bimodal one. The two groups thus demonstrated contain approximately equal numbers when pooled from the three muscles. Measurements at the other velocities gave essentially the same results. It seems likely that, as in the work of Rack &

Westbury (1966), the two groups correspond to primary and secondary afferents. Separation of units was clear in both pterygoid and temporalis muscles, but less complete in the masseter. The relative numbers of 'primary' and 'secondary' units were: masseter, 14:20; temporalis, 5:13 and pterygoid, 21:8 respectively.

DISCUSSION

Of the various cell types, thought at times to be present in the MeNV, we have been able to confirm the existence of only two. These are first order somata of tooth mechanoreceptors and of muscle spindles of the jaw-closing muscles. Though failure to find other cell types can never be regarded as totally satisfactory evidence for their absence, the extent of our search makes the presence of eye muscle proprioceptors, jaw-opening muscle proprioceptors or tendon organ afferents extremely unlikely.

It seems that in previous reports of eye muscle receptors (Cooper & Fillenz, 1955; Fillenz, 1955) confusion arose because of the disturbance of masticatory muscle spindles by movements in the orbit, which, in the cat, has no bony posterolateral wall. A wide variety of anatomical evidence summarized by Hosokawa (1961) suggests that eye muscle proprioceptors find their way into the ophthalmic branches of the fifth nerve. This is particularly well seen in the goat in which Whitteridge (1955) and Cooper & Daniel (1957) were able to find separate, purely sensory, nerve bundles passing from eye muscle nerves to fifth nerve branches in the orbit. This being so, there would be general grounds for expecting the afferent cell bodies to be in the trigeminal ganglion. There is direct evidence to this effect in the pig and sheep (Manni *et al.* 1966, 1968). In contrast, the sensory fibres which are well established to by-pass the ganglion and to have their cells in the MeNV, namely the jaw muscle proprioceptors (Corbin & Harrison, 1940; Szentagothai, 1948; Jerge, 1963) enter via the motor root of the fifth nerve (McIntyre, 1951). In the goat, in which evidence is probably best for the existence of eye muscle proprioceptor neurones within the brain stem (Cooper, Daniel & Whitteridge, 1953), responses were located close to the point of entry of the fifth nerve into the pons rather than in the MeNV. Moreover, the latency of 20–50 msec observed for the responses to eye muscle stretch cannot be accepted as good evidence of the cells concerned being first order. The present observations cause us to dismiss the representation of eye muscle proprioception in the MeNV of the cat and to have serious doubts about it in other species.

In most previous work on the MeNV, no special effort has been made to distinguish tendon organs from muscle spindles. Smith (1969) claimed to have shown increased discharge of stretch sensitive units during the rise

of tension in muscle twitches, but his stimuli were restricted to masseter and he did not appreciate that its contraction could excite spindles in the pterygoid muscle. The anatomical studies of Szentagothai (1948) argue against tendon organ representation in the MeNV since mid-brain lesions caused degeneration of spindle but not of tendon organ afferents. If, as would seem to be the case, the first order afferent cell bodies of tendon organs of jaw-closing muscles are not in the MeNV they would be expected to be in the trigeminal ganglion. The failure by Beaudreau & Jerge (1968) to find them there may have been due to the insensitivity of tendon organs to passive stretch of a relaxed muscle (Jansen & Rudjord, 1964). It would be desirable to look for tendon organ afferents again while trying to excite them with muscle twitches.

In the present work, initial attempts to characterize the jaw muscle spindle afferents as belonging to primary or secondary endings on the basis of their dynamic sensitivity were unsuccessful in the absence of fusimotor activation. Neither were vibration following nor variability of discharge of any real help in this situation. Conduction velocity measurements were not feasible, and in any case there is no certainty that the separation of fibres belonging to primary and secondary endings on this basis is possible except in cat hind-limb muscles. However, the histological work of Karlsen (1965) has shown the presence of typical spindles in rat jaw muscles with primary and secondary endings, and in the present work excitation of the spindles by SCh did lead to their clear division into two approximately equal groups on the basis of dynamic index. In the light of this finding, it seems unwise to attempt to separate primaries and secondaries functionally in other situations in the absence of fusimotor drive or of SCh activation. The inability of Koeze (1968) to find two populations of spindle afferents in the baboon tibialis anticus muscle is understandable in these terms.

As a result of this work, it is now fairly certain that all cells recorded in the MeNV can only belong to jaw-closing muscle spindles or tooth receptors. The latter are easily distinguished, so that we have a preparation in which spindle afferents are readily accessible to recording by extracellular electrodes without dissection and with a minimum of interference with the animal.

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The correlation of histochemistry and speed of contraction in cat jaw muscles

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The jaw-closing muscles of the cat have contraction speeds comparable with those of the extrinsic eye muscles (Taylor & Davey, 1968). We have studied their histochemical fibre types as an interesting case for comparison with the limb muscles.

Small pieces of temporalis, masseter and pterygoid, as well as samples of gastrocnemius and soleus muscles, were removed under pentobarbitone anaesthesia, immediately frozen in liquid-nitrogen-cooled iso-pentane, and cut in transverse section at 10 μ thickness. All specimens were stained for lipid (Sudan black), glycogen (PAS, McManus, 1946), myosin ATP-ase (Padykula & Herman, 1955), mitochondrial ATP-ase (Wachstein & Meisel, 1957) and succinic dehydrogenase (SDH, Nachlas, Tsou, Souza, Cheng & Seligman, 1957). The SDH reaction appeared to be the more reliable index of mitochondrial activity.

On the basis of the SDH and myosin ATP-ase reactions, two fibre types could be distinguished in each of the three jaw-closing muscles. The more plentiful type of fibre was large, stained weakly for SDH and strongly for myosin ATP-ase. A second type was smaller, and had converse staining properties. Lipid staining tended to follow SDH activity, whereas the PAS reaction was stronger in the large fibres.

In Table 1, data collected from seven cats show the relation between staining reaction and fibre size. The agreement in the estimates of fibre size of the two types distinguished by the myosin ATP-ase and SDH methods supports the reality of the distinction.

There seems little doubt that, on the basis of histochemistry and the speed of the whole muscle, the large fibres correspond to type A of gastrocnemius (Henneman & Olson, 1965), though in the jaw muscles these fibres are not so completely free of SDH staining. In the smaller fibres, ATP-ase staining is intermediate between that of B and C fibres in simultaneously prepared gastrocnemius, but the SDH reaction most resembles that of the C fibres.

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[P.T.O.

Table 1

	Myosin ATP-ase						SDH					
	No. (%)		Mean diameter (μm)		Mean cross-sectional area (μm^2)		No. (%)		Mean diameter (μm)		Mean cross-sectional area (μm^2)	
Muscle	D	L	D	L	D	L	L	D	L	D	L	D
Masseter	89·1	10·9	63·6 S.D.	39·7 S.D.	3240 S.D.	1260 S.D.	82·4	17·6	64·2 S.D.	39·1 S.D.	3310 S.D.	1230 S.D.
	n = 800		8·58 n = 50	5·33 n = 50	872	341	n = 296		10·5 n = 50	6·82 n = 40	1100	420
Pterygoid	72·9	27·1	75·7 S.D.	44·5 S.D.	4560 S.D.	1590 S.D.	61·2	38·8	78·5 S.D.	45·9 S.D.	4950 S.D.	1700 S.D.
	n = 1038		8·91 n = 50	6·56 n = 50	1060	459	n = 139		10·6 n = 50	5·89 n = 50	1350	469
Temporalis	97·8	2·2	71·2 S.D.	44·3 S.D.	4070 S.D.	1570 S.D.	89·5	10·5	71·1 S.D.	42·7 S.D.	4070 S.D.	1470 S.D.
	n = 639		10·6 n = 50	6·2 n = 50	1200	454	n = 239		10·8 n = 55	6·9 n = 36	1190	451

Fibres are distinguished as light staining (L) or dark staining (D).

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Histochemical and Mechanical Properties of the Jaw Muscles of the Cat

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The jaw-closing muscles, masseter and temporalis, in the cat reach peak isometric twitch tension in 11–13 msec and half relaxation from peak tension in 10–13 msec. They have high tetanus/twitch ratios and require stimulation at 300–500/sec to reach maximum rate of tension rise. In repeated tetani, some 75% of force is lost relatively rapidly by fatigue. Histochemical examination of serial sections stained for myosin ATPase (MATPase), succinic dehydrogenase (SDH), glyogen and lipid permits the fibers to be allocated to the recognized types A, B and C, with type A predominating. In the jaw muscles types A and C stain more strongly than type B for MATPase but are nevertheless paler than types A and C in gastrocnemius, and cannot be separated by this test alone. Jaw muscle type A fibers are somewhat richer in mitochondria than those of limb muscles. These observations are discussed in relation to the hypothesis that MATPase staining correlates best with speed and oxidative enzyme staining with fatigue resistance. It is concluded that the jaw-closing muscles form an interesting case for correlation of mechanical and histochemical properties because of the dominance of one (fast) fiber type. It is possible that the density of MATPase is not the only factor determining speed in these very fast muscles.

INTRODUCTION

Attempts to correlate the contraction characteristics and histochemistry of cat muscle fibers have been largely confined to hind limb muscles. On the basis of a combination of physiological properties, three distinct groups of motor units have been indentified in the gastrocnemius, whose histochemical profiles correspond to the three basic fiber types (9). Motor units designated FF (fast contracting, fast fatiguing) had high myosin adenosine triphosphatase (MATPase) and low mitochondrial oxidative enzyme activity; FR (fast contracting, fatigue resistant) units stained strongly for MATPase and moderately for oxidative enzymes; while S

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(slowly contracting, fatigue resistant) units were poor in MATPase and rich in oxidative enzymes. Thus the FF, FR and S motor units appear to resemble, respectively, the fiber types A, C and B described by Yellin and Guth (25) in cat muscle. This nomenclature is derived from the original classification of rat muscle fibers, according to succinic dehydrogenase (SDH) activity, of Stein and Padykula (23).

These findings suggest that the speed of contraction of individual fibers may be related to their MATPase activity while their mitochondrial content may be important in determining fatigability. The former hypothesis is supported by the proportionality of isometric twitch speeds and biochemically estimated MATPase activity in a variety of whole mammalian muscles (2). In addition cross-innervation experiments indicate that speeding of a muscle is accompanied by an increase in its MATPase activity (8) which is reflected by an increase in the percentage of its fibers staining strongly for this enzyme (12, 18, 22). That fatigability is related to mitochondrial content is consistent with the finding that fibers of fatigue resistant muscles, such as soleus, are relatively rich in mitochondria (15).

The jaw-closing muscles masseter, pterygoid and temporalis are known to be very fast, having contraction speeds approaching those of the extraocular muscles (24), and provide an interesting comparison with the slower limb muscles. The only previous report of jaw muscle histochemistry was in the rat (16). However, this study was based exclusively on Sudan black staining which does not permit reliable classification of fibers, and no parallel direct physiological recording was made from the muscles. In the present investigation we have examined the mechanical properties of the masseter and temporalis more fully and the histochemistry of these and of the pterygoid to see whether the above generalizations, relating histochemistry to function, apply here also. Some of the histochemical findings have been briefly reported (4).

METHODS

Mechanical Recording. Six adult cats of either sex, weighing 1.5–3.0 kg, were anesthetized with pentobarbitone sodium (60 mg/kg, ip) and maintained at a deep level by intravenous supplements. Isometric contractions were recorded from strips of masseter and temporalis, approximately 3 mm wide, taken from all regions of these muscles. The strips were separated from the bone at one end, being careful to leave the blood supply as intact as possible, and were tied to a strain gauge (Statham ± 24 oz). A skin pool filled with liquid paraffin maintained at 37–38 C surrounded the muscle. Stimulation was by indwelling enameled silver wires with their final 2 mm bared. The optimal muscle length for twitches, at

supramaximal stimulation, was determined and kept constant throughout recording.

Muscle twitches and tetani of 500 msec duration at increasing frequencies of 20–200/sec (pulse width 0.2 msec) were routinely recorded. In addition, in a limited number of preparations, the rate of rise of tetanic tension was measured at frequencies up to 600/sec.

Assessment of fatigability was made by the application of trains of stimuli at 55–90/sec, lasting 330 msec every 1 min. These parameters, allowing for the speed of the jaw muscles, were comparable to those employed in studying the fatigue susceptibility of motor units in limb muscles (9).

Histochemistry. Muscles were obtained from eight adult cats of either sex, weighing 1.5–2.0 kg. Small pieces of masseter, pterygoid and temporalis, as well as samples of gastrocnemius and occasionally soleus, were removed from different regions of these muscles under pentobarbitone anesthesia. These were blotted, immediately frozen in isopentane that had been cooled with liquid nitrogen and cut in transverse sections at 10 μm between −15 C and −20 C. All specimens were stained for MATPase (21), succinic dehydrogenase (SDH; 20), glycogen (PAS reaction; 19) and lipid (Sudan black). Cross-sectional areas of fibers were calculated from their mean diameters as measured with a micrometer eyepiece.

RESULTS

Mechanical Properties. Figure 1 illustrates some of the main properties of the masseter and temporalis muscles. Isometric twitches were fast. The mean times to peak twitch tension (T_P) for strips of masseter and temporalis were 13.1 (SD 2.27, $n = 18$) msec and 11.4 (SD 2.11, $n = 21$) msec, respectively. The corresponding times to half relaxation ($T_{\frac{1}{2}R}$) were 12.8 (SD 2.49) and 9.81 (SD 1.84) msec. In this paper T_c is defined as the time from the initial rise to the maximum twitch tension (P_0) and $T_{\frac{1}{2}R}$ as the time taken for P_0 to fall to half its value.

When stimulated at increasing frequencies tetanic fusion was complete at 100/sec. At greater frequencies there was negligible additional tetanic tension. However, the rate of rise of tension continued to increase at higher frequencies. There was an approximately linear relationship between the rate of rise of tension and log frequency up to 300/sec. Over the range 300–500/sec there was little further increase in the rate of tension development, which reached a maximum of 3–5% P_0/msec, and after 600/sec there were signs of a reduction. Tetanus:twitch (Tet:Tw) ratios were high for these muscles being 7.79 (SD 2.40, $n = 18$) for masseter and 9.60 (SD 4.06, $n = 20$) for temporalis. Neither mean contraction times

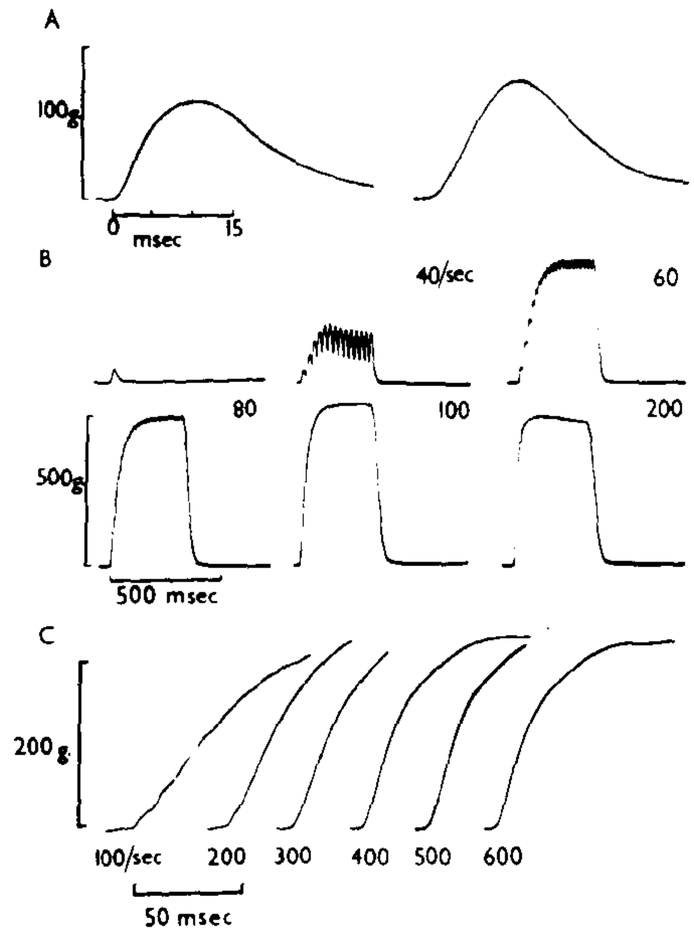

FIG. 1. A. Time course of isometric twitches of strips of masseter (left) and temporalis (right). The T_p was 10.0 and 11.5 msec, respectively. B. Tetanic stimulation of a strip of temporalis at increasing frequencies. Fusion is complete at 100/sec. The Tet: Tw ratio is approximately 11. C. The rate of rise of tetanic tension of a strip of temporalis at increasing frequencies of stimulation. A maximal rate of rise of 4.6% P_o/msec was obtained at 500/sec.

nor Tet: Tw ratios of the two jaw muscles were significantly different, as judged by the *t* test.

Post-tetanic potentiation was not great in these muscles, the twitch tension measured 10 sec after tetani of 500 msec durations at 100/sec was never more than 130% of the pretetanic tension.

Fatigue susceptibility was studied in a limited number of strips during the application of repetitive trains of stimuli. Typically there was a rapid fall in tetanic tension throughout the first minute of stimulation to about one-quarter of the initial level (Fig. 2). The force output of these muscles declined gradually over the next 2–3 min. The effect of more prolonged stimulation was not tried.

Histochemistry. In the jaw-closing muscles three fiber types, essentially similar to the A, B and C fibers of hind limb muscles, were classified on the basis of a combination of their MATPase, SDH and PAS staining. However, the jaw muscle fiber types differed from the corresponding limb muscle fiber types in certain aspects of their histochemistry, especially MATPase staining, and in their relative numbers.

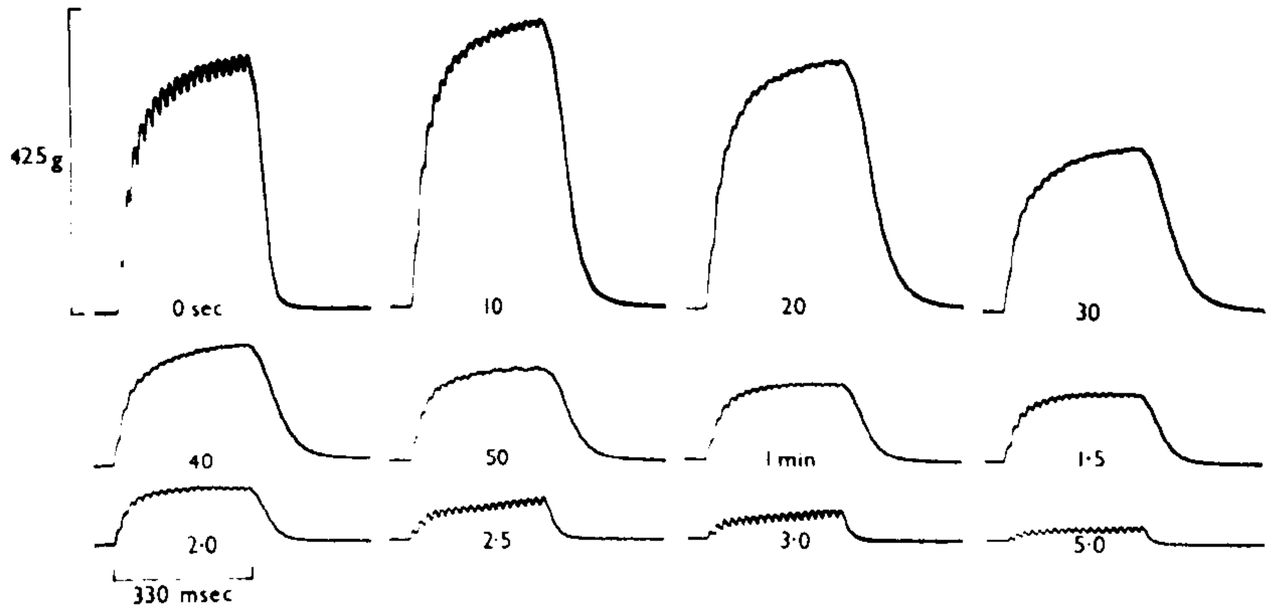

FIG. 2. Fatigue of tetanic tension of a strip of temporalis muscle during repetitive stimulation at 55/sec.

Individual fibers of each jaw muscle were identified in adjacent serial sections stained in parallel for MATPase, SDH and glycogen (Figs. 3 and 4). The classification is based primarily on the SDH staining. Sections of gastrocnemius stained at the same time are included for comparison.

The predominant type of fiber was large. The MATPase staining was relatively strong, enzyme activity being even throughout the fibers. The SDH reaction was weak, suggesting that few mitochondria were present, with diformazan particles being somewhat concentrated peripherally. The PAS staining indicated that the glycogen content of these fibers was high. This fiber type resembled the A fibers of gastrocnemius.

A second fiber type was of intermediate size. It showed MATPase activity similar to that of the A fibers and was rich in glycogen. The SDH staining was strong, with a marked, although often discontinuous, preferentially peripheral distribution of coarse diformazan particles. This indicated that the mitochondrial content was high and mainly subsarcolemmal in location. This fiber type resembled the C fibers of gastrocnemius.

A minority of fibers were small, stained weakly for MATPase and were poor in glycogen. Their SDH reaction was well developed, with small diformazan particles being evenly scattered throughout the cytoplasm. This fiber type most closely resembled the B fibers of gastrocnemius.

Sudan black staining was comparable to SDH activity but was far less distinct.

The cross-sectional areas and relatives numbers of individual fibers, classified as A, B or C by their histochemical profile, were measured and are presented in Table 1. In each of the jaw muscles, each fiber type was

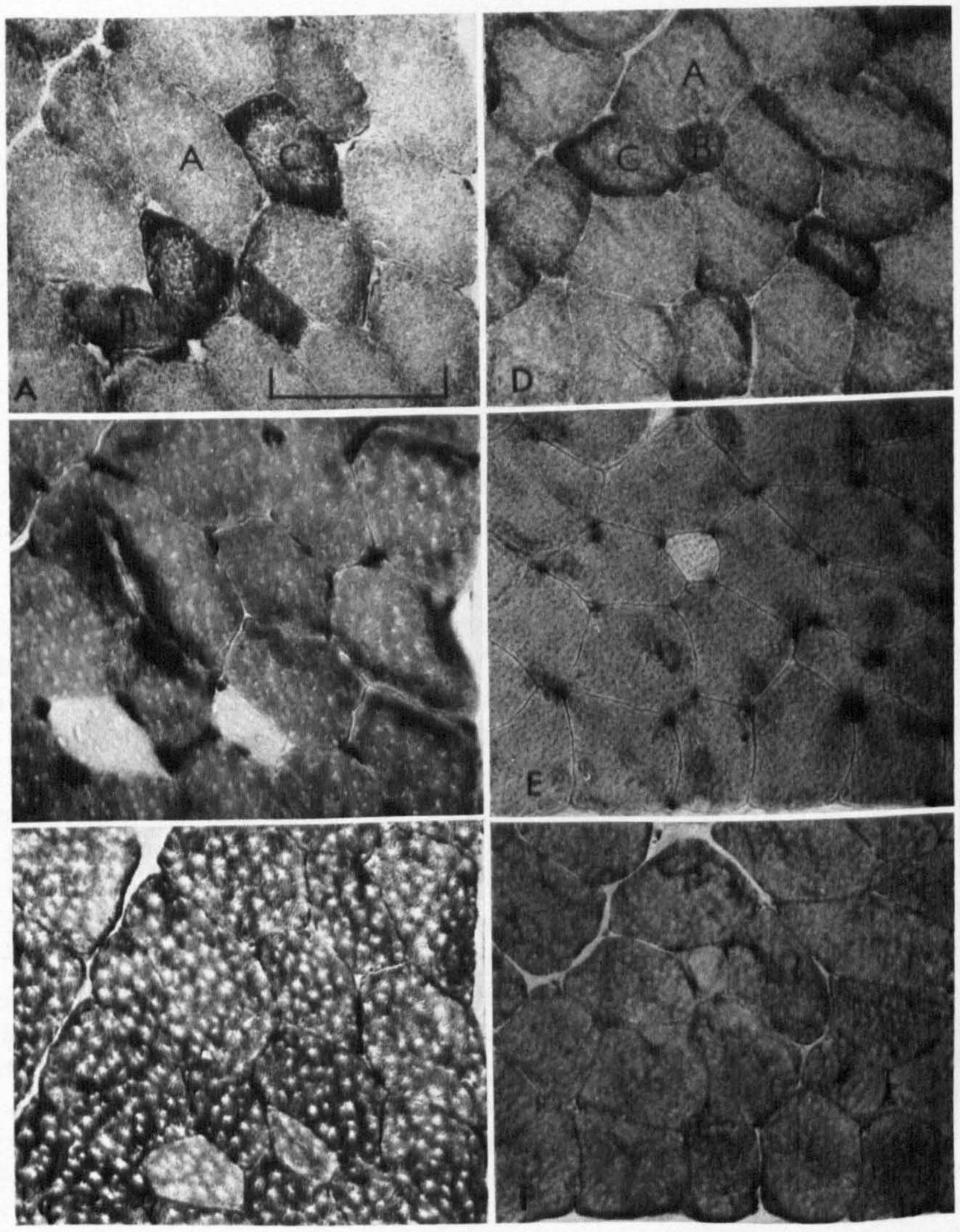

FIG. 3. The histochemical reactions of individual fibers identifiable in adjacent sections. A. Masseter, SDH. B. Masseter, MATPase. C. Masseter, PAS. D. Temporalis, SDH. E. Temporalis, MATPase. F. Temporalis, PAS. The calibration bar is 100 μm for all photomicrographs.

significantly different from either of the others in mean cross-sectional area according to the t test ($P = 0.001$).

A difference noticed between the jaw and limb muscles concerned their MATPase staining. Whereas the A, B and C fibers of gastrocnemius were

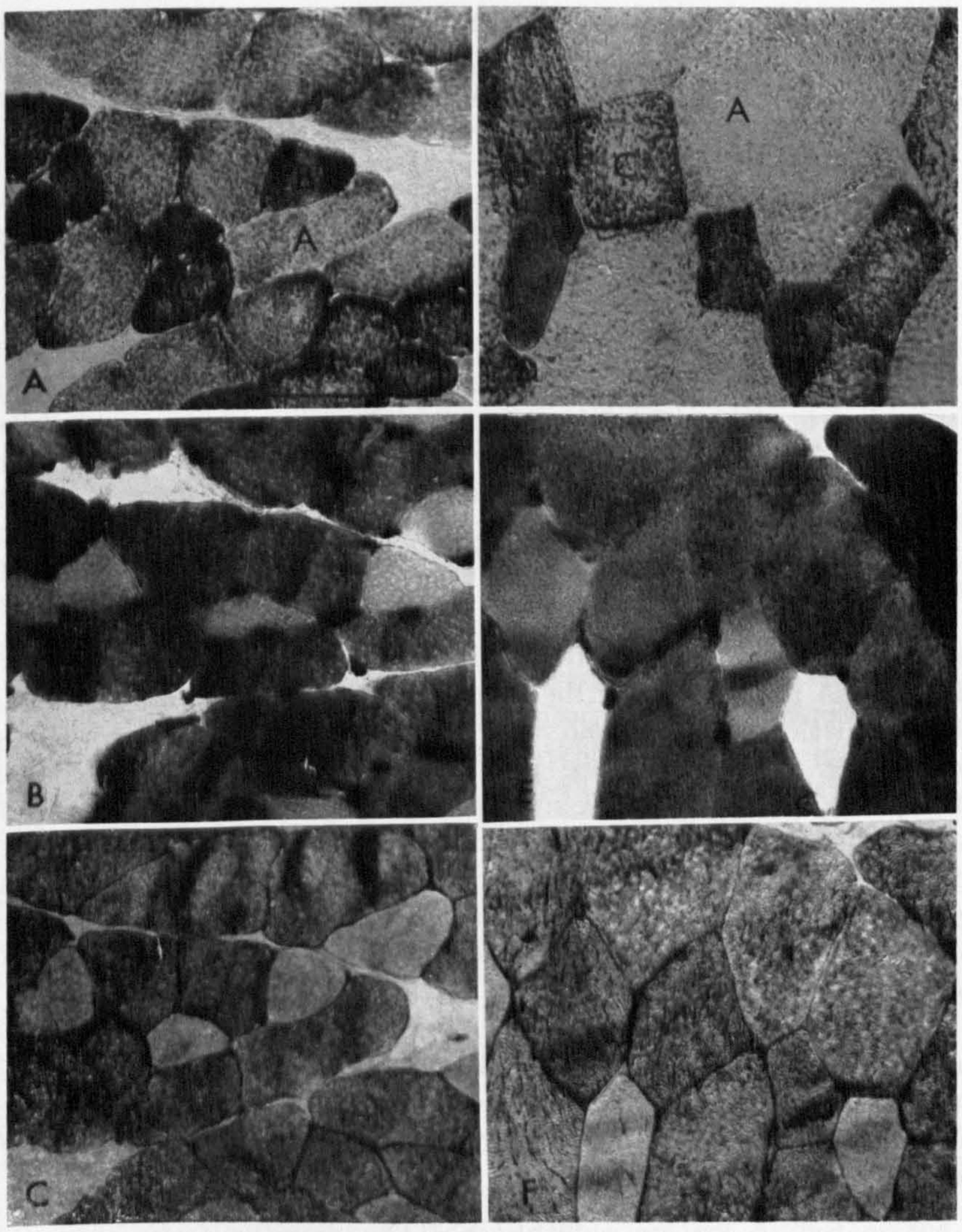

FIG. 4. The histochemical reactions of individual fibers identifiable in adjacent sections. A. Pterygoid, SDH. B. Pterygoid, MATPase. C. Pterygoid, PAS. D. Gastrocnemius, SDH. E. Gastrocnemius, MATPase. F. Gastrocnemius, PAS. The calibration bar is 100 μm for all photomicrographs.

separable according to the intensity of their reaction, at pH 9.4, (Fig. 4), in the jaw muscles the A and C fibers were indistinguishable. Consequently only two sorts of fibers were revealed in the jaw muscles by this method. Also, unexpectedly, this reaction was consistently less developed in the

TABLE 1

NUMBERS AND SIZES OF FIBER TYPES IN JAW MUSCLES[a]

Muscle	TYPE A %	TYPE A Mean cross-sectional area (μm^2)	TYPE B %	TYPE B Mean cross-sectional area (μm^2)	TYPE C %	TYPE C Mean cross-sectional area (μm^2)
Masseter	82	4100 (SD 1210)	10	1400 (SD 809)	8	2670 (SD 545)
Pterygoid	51	3380 (SD 840)	29	1300 (SD 447)	20	2330 (SD 463)
Temporalis	72	3770 (SD 826)	2	871 (SD 284)	26	2590 (SD 774)

[a] In each muscle estimates were based on 200 fibers for which full histochemical profiles were obtained.

jaw muscles than in the gastrocnemius with which they were incubated. Thus the A and C fibers of the former group were somewhat paler than their equivalents in the limb muscle. The SDH reaction was almost identical in fiber types of the jaw and limb muscles, differing only in that the A fibers of the jaw muscles were slightly less free of enzyme activity. In neither the jaw nor the limb muscles could fiber types be readily categorized by their glycogen or lipid content. The PAS reaction was generally greater in the A and C fibers than in the B type, whereas Sudan black staining, while following SDH activity, was never as clear an indicator of mitochondrial distribution.

The jaw muscle fibers had an excellent blood supply, with each type of fiber commonly surrounded by three or four capillaries. In the gastrocnemius there were fewer blood vessels and a gradation in the number associated with each fiber type, being greatest for B fibers and least for A fibers.

DISCUSSION

The cat jaw-closing muscles, masseter and temporalis, were found to be fast, confirming the earlier observations of Taylor and Davey (24). Twitch speeds were intermediate between those of the extraocular muscles (1, 11) and the most rapidly contracting hind limb muscles, e.g., flexor hallucis longus (5). In common with other muscles having short twitch times, such as the extraocular muscles and the thyroarytenoid (17), the Tet:Tw ratio was high, being almost twice that reported for limb muscles (6). Apparent fusion and maximal tetanic tension was obtained at 100/sec.

However, as demonstrated in other muscles, the rate of rise of tetanic tension continued to increase at higher frequencies, and reached a maximum at 400–500/sec. The maximal rate of rise of tension and the frequency necessary to achieve this were comparable in the jaw muscles and FHL whereas the corresponding values are lower in the slow soleus (7). In the extraocular muscles high frequencies of stimulation have recently been shown to be especially important in obtaining the maximal rate of rise of tension; the latter increases considerably between 400 and 600/sec (3, 13).

Fatigue of the jaw muscles, during repetitive trains of stimuli, occurred in two phases. Initially tetanic tension declined quickly to a level which thereafter showed only a small further fall. A possible interpretation is that the early reduction in tension is due to rapid fatigue of one group of motor units, while the remainder are relatively insusceptible and maintain their force output for longer periods. This interpretation is consistent with single motor unit studies in gastrocnemius in which FF units showed early fatigue, while the tetanic tension of FR units was only slightly reduced over the first 6 min of stimulation and that of S units was unchanged for up to 1 hr (9).

In the jaw-closing muscles the A and C fibers together constituted over 95% of the cross-sectional area, a higher percentage than in the gastrocnemius. These two fiber types had similar MATPase staining, at pH 9.4, having greater enzyme activity than the minority B fibers. Therefore these muscles would appear to be an especially suitable group in which to attempt to correlate speed of contraction and MATPase activity, since the contraction characteristics of whole muscles must represent those of the predominant fibers.

That the majority of fibers were relatively high in MATPase activity is consistent with the hypothesis that this enzyme is important in determining the speed of contraction. However, this is complicated by the finding that the intensity of staining of the A and C fibers of the jaw muscles was less than that of their equivalents in the gastrocnemius. In the limb muscles A and C fibers appear to form, respectively, FF and FR motor units which although rapidly contracting are not as fast as the jaw muscles (9).

A possible explanation is that the MATPase in the fast jaw muscle fibers is not the same as that in limb muscles. A body of evidence, outlined by Close (10), indicates that the myosins of fast and slow limb muscles are chemically distinct proteins with different specific MATPase activities. Guth and his co-workers (14) have demonstrated histochemically that the MATPase of the various fiber types differ in pH stability. Thus the unexpectedly low MATPase staining of jaw muscle fibers vis-a-vis

hind limb fibers could result from a difference in alkali stability of the enzyme. The histochemical demonstration of Ca^{2+} activated MATPase must be done at the artificial pH of 9.4, in order to prevent solubilization of the reaction product. At physiological pH this method does not work well. It seems unwise therefore to attempt quantitative comparisons between MATPase activities measured under these conditions since it is difficult to interpret their relationship to those present *in vivo*.

However, a relatively high MATPase is not necessarily the rate-limiting factor determining the speed of the jaw muscles. The kinetics of excitation–contraction coupling may also play a part in defining the time course of very fast twitches. In particular the time course of the active state is now widely believed to depend on the rate of exchange of Ca^{2+} ions between the sarcoplasmic reticulum and the sarcoplasm. In this context a study of the sarcoplasmic reticulum of very rapidly contracting muscles would be interesting.

The division of fibers into those poor (type A) and those rich in mitochondria (types B and C) is similar to that expected if mitochondrial content determined fatigability. Rapid fatigue of the predominant A fibers could account for the large initial fall in tetanic tension observed, while B and C fibers, fatiguing more slowly could account for the second phase. However, at present little can be said about the minority types of fibers in the jaw muscles other than that they are presumably fatigue resistant. Single unit studies would be required to confirm these speculations.

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The behaviour of spindles in the jaw-closing muscles during eating and drinking in the cat

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Enough is now known of the detailed function of mammalian muscle spindles, studied in isolation, to permit the formulation of various theories of their action in controlling voluntary movement. Direct testing of such theories demands recording from muscle spindle afferents during normal movements, unaffected by anaesthesia. Hagbarth & Vallbo (1968) and Vallbo (1971) have recently begun to obtain such data from human nerve

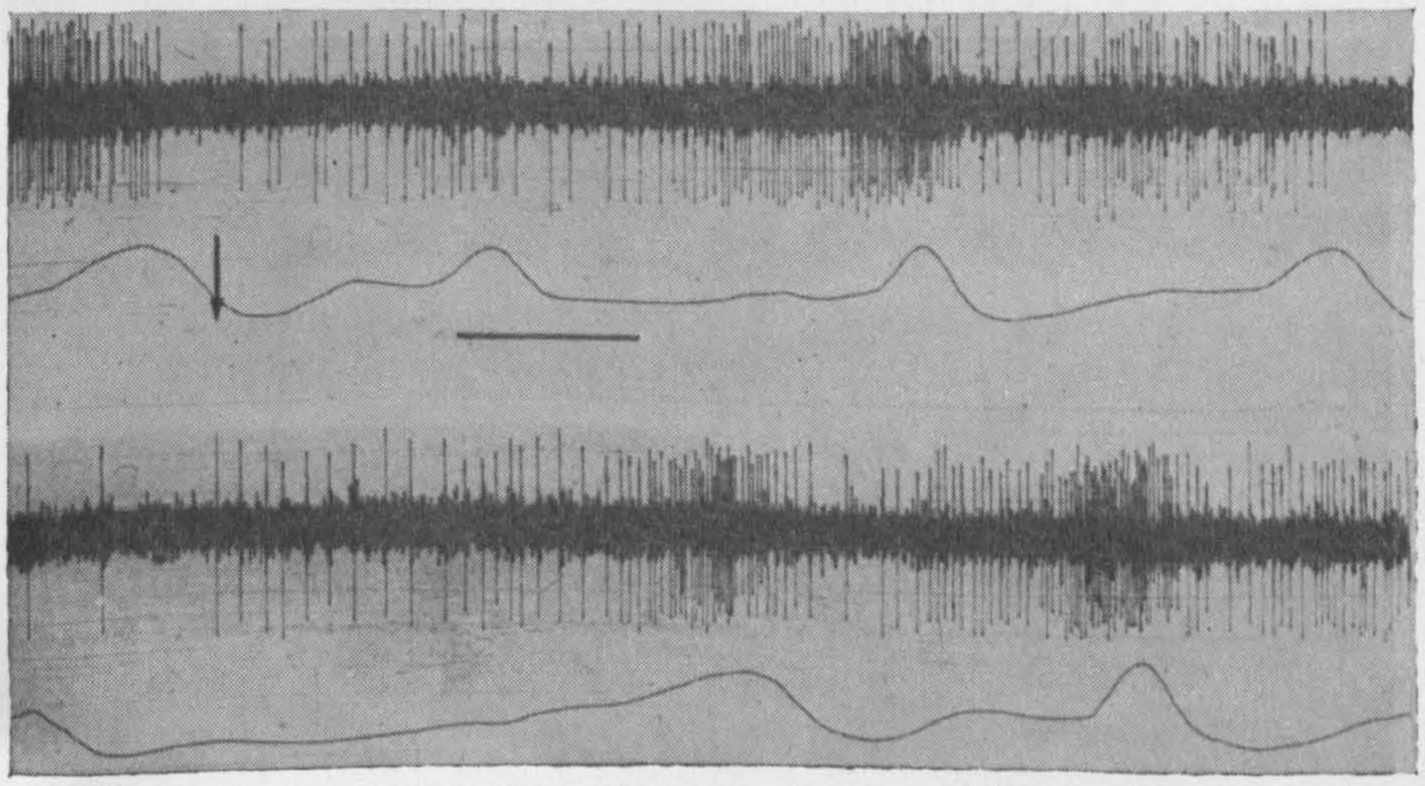

Fig. 1. Responses of a single jaw closing muscle spindle afferent unit (upper trace) and jaw displacement (lower trace), recorded during eating. The arrow represents 25° of jaw closing and the time bar is 200 msec.

recording during isometric contractions and during certain restricted movements. We have approached the same problem in the conscious cat by recording from 1st order cells belonging to jaw-closing muscle spindles. These are known to be located in the mesencephalic nucleus of the fifth cranial nerve (Cody, Lee & Taylor, 1972). At the same time, jaw movements have been recorded (Taylor, 1969) together with e.m.g. activity from the masseter and temporalis.

Single unit records obtained during eating (Fig. 1) show that spindle discharge rises to high frequencies during the jaw opening phase. It then

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decreases during active shortening of the muscle, when it is often silenced. On some occasions (during licking the lips), jaw movements of 25° could occur with virtually no change in spindle frequency. The pattern of activity during eating was very variable, though the movements were quite stereotyped. During the rhythmic movements of lapping, spindle discharge showed a more constant pattern.

The fact that the spindle firing does not increase during active muscle shortening shows that the spindle afferents cannot be driving the movement reflexly, as would be required by the 'length follow-up servo' hypothesis expressed by Merton (1953). This does not imply that there is no fusimotion under these conditions. Indeed, it is evident on comparing discharge during active and passive movements (Taylor & Davey, 1968) that phasic fusimotor activity must have been going on parallel with α motor neurone excitation, during the natural movements described.

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